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Original paper

Evaluation of the relationship between the geriatric anxiety and COVID-19 anxiety and fear levels in geriatric dental patients during the COVID-19 pandemic

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Background. Illness, social isolation and loneliness may cause different psychological problems in the geriatric population, including depression and anxiety. Factors such as anxiety and fear can negatively affect dental treatment processes and prognoses. Thus, in terms of dental approach to geriatric individuals, it is important to be aware of the emotional processes the elderly may have experienced during the pandemic.

Objectives. This study aimed to determine the relationship between the geriatric anxiety levels and the coronavirus disease 2019 (COVID-19) anxiety and fear levels in the geriatric population.

Material and methods. In this correlational study, 129 geriatric individuals were selected through the convenience sampling method. To gather the data, the Geriatric Anxiety Scale (GAS), the COVID-19 Anxiety Scale (CAS), the COVID-19 Fear Scale (CFS), and a questionnaire assessing demographic variables were used. Simple linear regression and Pearson's correlation coefficients were used to evaluate the relationships between the variables.

Results. The sample consisted of 70.5% males and 29.5% females aged ≥65 years. The GAS total score (15.64 ±9.34) and its 3 subscale scores were strongly correlated with the CAS and CFS scores. The GAS total score and its subscale scores had a significant linear regression with both the CAS and CFS scores ($p < 0.001$).

Conclusions. An increase in the anxiety and fear levels associated with the pandemic was observed in geriatric individuals. Thus, it should be considered that geriatric individuals may encounter some difficulties during dental treatment and prosthetic rehabilitation after the pandemic. Therefore, it is important to normalize the anxiety levels with the help of professionals, and to implement interventions such as socialization, physical activity and meditation to help balance the anxiety levels.

Keywords: coronavirus disease, COVID-19 pandemic, fear, geriatric anxiety

Introduction

Senility is a physiological process that reduces or limits the level of physical activity, increases the occurrence of diseases that require drug use, weakens cognitive functions, and thus makes people socially, physically and emotionally dependent to varying degree.[1,2] In addition to a gradual decrease in the ability to adapt to environmental factors, the senility limit is defined as being over 65 years old in most developed western countries, over 60 years old by the United Nations, and over 65 years old by the World Health Organization (WHO).[1] With aging, regression occurs in both the immune system and the anatomical and physiological natural defense systems against pathogens. Infectious diseases are more common in geriatric individuals and they can be more severe than in young people.[3–5] Coronavirus disease 2019 (COVID-19) is not fatal in the majority of the elderly. However, a significant number of the patients who died from COVID-19 and needed intensive care were elderly individuals. Considering this situation, a fear of getting the disease and of infecting relatives and peers with the disease, and an increase in the anxiety levels can be observed in elderly individuals.[6,7] In addition, the social isolation and loneliness caused by the pandemic are dangerous to the geriatric population, and may cause psychological effects due to increased dependence on others with advancing age.[8] Social media, and electronic and printed media articles about the COVID-19 pandemic can cause strong emotions, such as anxiety and fear.[9] It has been reported that two of the most important factors negatively affecting quality of life in the elderly population are anxiety and depression, and that these conditions are responsible for an increase in the death rate.[10,11] Fear is an emotional reaction triggered by a known danger or threatening situation that causes behaviors such as the fight-or-flight response.[9] Anxiety, which can occur at different intensities, is defined as a feeling of uneasiness toward an unknown danger.[12] To understand the psychological repercussions of the COVID-19 pandemic, emotions such as anxiety and fear should be taken into account and closely observed.

Partial and total tooth loss is common in the geriatric population. Thus, prosthetic dentistry plays an important role in restoring the function and esthetics of these edentulous patients. The psychological states and moods of patients significantly affect both the treatment processes and patient satisfaction with the treatment results.[13–15] According to Berggren and Meynert, if an individual has a predisposition to anxiety, a vicious circle of fear, anxiety-induced avoidance of dental care, deterioration of dentition, and feelings of guilt, shame and inferiority can occur.[16] In anxiety disorders, the main cause of physical symptoms is thought to be the sympathetic nervous system stimulation.[17] The stimulation of the autonomic nervous system may cause tachycardia, arterial hypertension, dyspnea, perspiration (especially of the palms), a tight throat, light-headedness, a dry mouth, nausea, and a decreased pain threshold.[18,19] In addition, other studies have reported that COVID-19 causes symptoms such as anosmia, loss of taste, headache, and rhinosinusitis.[20–22] These symptoms may disrupt the patient's compliance during treatment, make the patient feel more pain, impede the dentist's work, and increase nausea, all of which may complicate the impression process and result in an unpleasant dental experience.[23] Thus, it is important to evaluate the anxiety levels in the approach to patients. Several studies have evaluated the psychological effects of the COVID-19 pandemic on doctors, nurses and caregivers, and have reported increased fear, anxiety and stress during the pandemic.[24–27] It has also been observed that fear and anxiety differ between individuals, and that these differences are affected by sociodemographic variables.[28] Although there are studies evaluating anxiety and fear in geriatric individuals before and during the COVID-19 pandemic, there are no studies evaluating these effects during the controlled normalization phase, which followed the withdrawal of the stay-at-home and masking orders. Thus, the present study aimed to determine the relationship between the geriatric anxiety levels and the COVID-19-induced anxiety and fear levels in geriatric individuals.

Methods

A cross-sectional online survey was conducted with the participation of geriatric individuals. An age of 65 years was accepted as the senility limit, and individuals aged 65 and above were included in the study. The survey was conducted between March 1 and March 31, 2021. During this period, controlled normalization was started in Turkey and most pandemic restrictions continued. Prior to starting the study, an approval (2022/38) was obtained from the Ethics Committee at the Faculty of Dentistry of Ataturk University, Erzurum, Turkey. The study was carried out in accordance with the 2008 Declaration of Helsinki, and all participants signed an informed consent form prior to participation. A total of 129 geriatric patients aged 65 and above, who previously had visited the Ataturk University Faculty of Dentistry and had been reached through social media platforms, agreed to fill out the online questionnaire. The questions were prepared in Turkish and included both multiple-choice and close-ended questions. The online survey was designed through https://docs.google.com/forms and sent to the participants via social media and e-mail. The questionnaire consisted of 4 main parts. In the 1st part, 4 out of 8 questions evaluated sociodemographic characteristics (age, gender, the educational level, and chronic illnesses). The 2nd, 3rd and 4th parts of the questionnaire included the Geriatric Anxiety Scale (GAS) consisting of 30 questions,[29] the COVID-19 Anxiety Scale (CAS) consisting of 5 questions[30] and the COVID-19 Fear Scale (CFS) consisting of 7 questions, respectively.[31]

The GAS is a 30-item self-report scale used to measure anxiety symptoms, especially among older adults.[29] Individuals are asked to indicate how often they have experienced each symptom over the past week, including the day of questioning. The questions are answered using a 4-point Likert scale ranging from 0 (not at all) to 3 (all of the time). The GAS total score is based on the first 25 items. There are 3 subscales: somatic (9 items); cognitive (8 items); and affective (8 items). The last 5 items are used by clinicians to determine the area of anxiety. These items cannot be included in the total score of this scale or its subscale scores.[32] Scores range from 0 to 75, and higher scores indicate greater anxiety.

The CAS was developed by Lee[33] and adapted to Turkish by Evren et al.[30] It consists of 5 questions asking participants about how often they have experienced the situations presented in the statements on the scale over the last 2 weeks. Participants are asked to use a 5-point time-anchored scale ranging from 0 (not at all) to 4 (nearly every day over the last 2 weeks). While the maximum score that can be obtained on the scale is 20, the minimum score is 0. The optimized cutoff score was accepted as 9, and it was determined that participants with high scores had high COVID-19 anxiety levels.[30]

The CFS was developed by Ahorsu et al.[34] and adapted to Turkish by Haktanir et al.[31] Participants are asked to answer 7 questions using a 5-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). The maximum score that can be obtained on the scale is 35, and the minimum score is 7.[31] The optimized cutoff score was accepted as 17.5, and it was determined that participants with high scores had high COVID-19 fear levels.[35]

Statistical analysis

The obtained data was analyzed using the IBM SPSS Statistics for Windows program, v. 22 (IBM Corp., Armonk, USA). The categorical variables are presented as number and percentage (n (%)), and the numerical variables are presented as mean and standard deviation ($M \pm SD$). The adherence of the numerical variables to a normal distribution was investigated using the Kolmogorov–Smirnov test. For the statistical evaluations, $p < 0.05$ was considered statistically significant. The distribution of the data was parametric, and simple linear regression and Pearson's correlation coefficients were used to evaluate the correlations between the variables.

Results

A total of 129 patients – 91 (70.5%) males and 38 (29.5%) females – were included in the study. The sociodemographic characteristics of all participants and additional information about COVID-19 are presented in Table 1. The mean scores for the GAS were as follows: GAS total score –15.64 ±9.34; somatic subscale score – 6.17 ±3.56; cognitive subscale score – 3.85 ±3.41; and affective subscale score – 5.62 ±3.47. The mean scores for the CAS and the CFS were as follows: CAS score – 7.67 ±2.75; and CFS score – 15.6 ±5.55. The GAS total score and the 3 subscale scores were significantly correlated with the CAS and CFS scores, as shown in Table 2. As shown in Table 3, the GAS total score and the 3 subscale scores had a significant linear regression with the CAS score ($p < 0.001$). Similarly, the GAS total score and the 3 subscale scores had a significant linear regression with the CFS score ($p < 0.001$), as shown in Table 4.

Table 1. Sociodemographic characteristics of the participants and participant information about coronavirus disease 2019 (COVID-19)

Sociodemographic characteristics and COVID-19 data		n (%)
Age [years]	65–69	60 (46.5)
	70–74	49 (38.0)
	75 and above	20 (15.5)
Gender	M	91 (70.5)
	F	38 (29.5)
Educational level	primary school	27 (20.9)
	secondary school	3 (2.3)
	higher school	13 (10.1)
	graduate	56 (43.4)
	postgraduate	30 (23.3)
Chronic illnesses	hypertension	63 (48.8)
	diabetes mellitus	16 (12.4)
	asthma	7 (5.4)
	chronic obstructive pulmonary disease	3 (2.3)
	chronic kidney disease	6 (4.7)
	coronary artery disease	19 (14.7)
	other	7 (5.4)
	none	8 (6.2)
State of being infected with COVID-19	I did not have the disease	103 (79.8)
	I had the disease once	24 (18.6)
	I had the disease twice	2 (1.6)
Vaccination status	0	2 (1.6)
	1 dose	0 (0.0)
	2 doses	14 (10.9)
	3 doses	32 (24.8)
	4 doses	81 (62.8)
Reason for getting vaccinated	a fear of COVID-19 complications	38 (29.5)
	a fear of COVID-19 complications due to advanced age	28 (21.7)
	a fear of COVID-19 complications due to a chronic illness	17 (13.2)
	protecting public health	44 (34.1)
Reason for not getting vaccinated	a fear of vaccine side effects	2 (1.6)
	the thought that the vaccine will not be useful	2 (1.6)
	a fear of being negatively affected by the vaccine because of a chronic illness	1 (0.8)

N = 129; M – male; F – female.

Table 2. Correlation matrix for the COVID-19 Anxiety Scale (CAS), the COVID-19 Fear Scale (CFS) and the Geriatric Anxiety Scale (GAS) (total and subscales)

Scale	GAS total	GAS somatic subscale	GAS cognitive subscale	GAS affective subscale	CAS	CFS
CAS	0.429*	0.406*	0.370*	0.374*	–	0.442*
CFS	0.493*	0.454*	0.430*	0.439*	0.442*	–

* $p < 0.001$ (two-tailed).

Table 3. Regression analysis of the effect of the COVID-19 Anxiety Scale (CAS) score on the Geriatric Anxiety Scale (GAS) total score and its subscale scores

Predictor	Outcome	*R*	R^2	*t*	*β*	*p*-value
CAS score	GAS total score	0.429	0.184	5.353	0.429	<0.001*
	GAS somatic	0.406	0.165	5.008	0.406	<0.001*
	GAS cognitive	0.370	0.137	4.490	0.370	<0.001*
	GAS affective	0.374	0.140	4.543	0.384	<0.001*

* statistically significant.

Table 4. Regression analysis of the effect of the COVID-19 Fear Scale (CFS) score on the Geriatric Anxiety Scale (GAS) total score and its subscale scores

Predictor	Outcome	*R*	R^2	*t*	*β*	*p*-value
CFS score	GAS total score	0.493	0.243	6.393	0.493	<0.001*
	GAS somatic	0.454	0.206	5.745	0.454	<0.001*
	GAS cognitive	0.185	0.179	5.371	0.185	<0.001*
	GAS affective	0.439	0.192	5.502	0.439	<0.001*

* statistically significant.

From the sociodemographic data, gender ($\beta = -0.44$; $t = -5.35$; $p < 0.001$) and the educational level ($\beta = -0.25$; $t = -3.02$; $p = 0.003$) had a significant linear regression with the GAS total score (F (2,121) = 32.455; $p < 0.001$; $R^2 = 0.35$) ($p < 0.001$). Gender ($\beta = -0.29$; $t = -3.40$; $p = 0.001$) and the educational level ($\beta = -0.33$; $t = -3.88$; $p < 0.001$) had a significant linear regression with the CFS score (F (2,121) = 23.904; $p < 0.001$; $R^2 = 0.28$) ($p < 0.001$). Gender ($\beta = -0.21$; $t = -2.10$; $p = 0.018$), the educational level ($\beta = -0.36$; $t = -4.14$; $p < 0.001$) and the presence of a chronic illness ($\beta = 0.17$; $t = 2.15$; $p = 0.033$) had a significant linear regression with the CAS score (F (3,120) = 16.171; $p < 0.001$; $R^2 = 0.29$) ($p < 0.001$). Other sociodemographic data did not show a significant linear regression with the scales ($p > 0.05$).

Discussion

The main source of anxiety in old age is concern about physical health. Anxiety symptoms among the geriatric population have been associated with a variety of psychological outcomes, including depression, social isolation and loneliness. In this study, the relationships between the COVID-19 anxiety and fear levels and the geriatric anxiety levels in geriatric individuals were examined, and it was revealed that there were significant positive relationships between these variables. At the same time, the results of this study showed that there were significant positive relationships between the geriatric anxiety subscales (psychosomatic and emotional) and the COVID-19 anxiety and fear levels.

In this study, greater anxiety and fear levels were observed in females and less educated individuals. Other studies without age restrictions have reported similar results. For example, Wieckiewicz et al. found a greater psychological impact of the pandemic and increased anxiety levels in females aged less than 28.5 years who were less educated.[21] In other studies, the psychological effects of the pandemic have also been observed to be greater in less educated female individuals.[36,37] Based on the results cited above, it appears that the pandemic has affected to a greater extent less educated females, regardless of age.

During the COVID-19 pandemic, several studies examined the COVID-19-induced anxiety and fear levels in dentists, postgraduate students in dentistry, and in individuals with different sociodemographic characteristics.[38–40] Geriatric individuals, who are thought to be more affected by the emotional damage caused by the pandemic, were included in this study, as the treatment processes and the satisfaction of patients with prostheses could be affected by increased anxiety levels. There have been several COVID-19-related studies on geriatric individuals. For example, Balasundaram et al. associated the fear of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection with the psychological distress and anxiety caused by curfew, which negatively affected the healthcare and non-COVID-19 medical services of the elderly.[41] Mowla et al. found high rates of depression and anxiety symptoms in their comparative study on elderly people who had not had the disease during the pandemic and those who had survived COVID-19.[42] Köverová et al. examined the levels of stress, general anxiety and COVID-19-related anxiety during the 1st and 2nd waves of the pandemic, and highlighted the roles of the psychological predictors of stress, anxiety and COVID-19 anxiety during both waves.[43] Bergman et al. evaluated the moderating role of COVID-19-related ageism in the relationship between COVID-19 health worries and anxiety symptoms among geriatric people, and showed that both health worries and ageism were positively associated with anxiety symptoms.[44] Unlike these studies conducted during the COVID-19 pandemic, our study examined the levels of anxiety and fear related to COVID-19 as well as the geriatric anxiety levels during the normalization phase of the pandemic. As such, this study was designed in a different way; however, it should be noted that previous studies suggested the use of the GAS after the pandemic.

For geriatric individuals living alone, the presence of family members and the visits of relatives are an important source of social interaction and joy. The social distancing measures implemented due to the COVID-19 pandemic forced people to stay in their homes and to limit contact with the outside world. This caused social isolation among geriatric individuals and great concern for their own and their relatives' safety.[45] Studies have shown that the health risks related to social isolation can be equal to those related to smoking and obesity.[46] During isolation, the needs for socialization, religious/spiritual services, medical assistance, home delivery, and therapy are often met through the Internet. However, as older adults are not typically skilled at or comfortable using technology and online platforms, they probably did not benefit from any of the services offered. Partly due to these conditions, the geriatric population is at higher risk of suffering from anxiety.[8] In our study, despite the pandemic and associated restrictions winding down, geriatric individuals were observed to have high anxiety and fear levels related to COVID-19. The lack of a decrease in both the general anxiety levels and the COVID-19 anxiety and fear levels can be explained by the fact that traces of these traumatic effects are still seen after the pandemic. Barg et al.[47] and de Beurs et al.[48] found significant positive associations between loneliness and anxiety in older adults, and Khademi et al., in a study conducted in Iran, reported a high level of correlation between loneliness and the anxiety levels.[49] In the current study, a significant correlation was found between the COVID-19 anxiety and fear levels and the geriatric anxiety levels. These associations may be related to the loneliness caused by social isolation during the COVID-19 pandemic.

The lowest mean GAS total score (10.1 ±6.8) was reported in a study conducted in the USA and Canada.[50] The scores found in other countries and studies are as follows: in the USA – 13.65 ±9.70[29]; in Italy – 13.08 ±7.95[51]; and in Germany – 10.51 ±8.95.[32] The mean GAS total scores obtained in studies conducted in Turkey[52] and Iran[49] are consistent with the current results (18.01 ±12.78 and 17.12 ±12.34, respectively). These results suggest that the anxiety levels in geriatric individuals living in Eastern societies are higher than in Western societies. Although the abovementioned Turkish GAS study[52] was conducted before the pandemic, the GAS total score was found to be higher than in our study. The reasons for this difference in scores may be due to regional differences with regard to the places where the studies were conducted.

Limitations

As in all studies, there are some limitations to the current research. First, the results are based on the analysis of cross-sectional self-reported data, which does not allow causal inference, and bias due to the common-method variance might occur. There is also an imbalance between the number of male and female participants, with females being underrepresented in this sample. In addition, there are many factors that affect the anxiety and fear levels. The fact that the unmeasurable factors regarding the effects of the pandemic were not addressed is another limitation of the study. As this study is correlational, it cannot be proven whether one variable causes a change in the other. Thus, further research on more individuals is needed to better understand the relationships between the different types of anxiety.

Conclusions

In our study, an increase in the anxiety and fear levels caused by the COVID-19 pandemic was observed in geriatric individuals in the high-risk group. Hence, it should be considered that geriatric individuals may encounter some difficulties during dental treatment and prosthetic rehabilitation after the pandemic. Therefore, it is important to normalize the anxiety levels in these patients. Due to the pandemic, most of these individuals will probably need interventions or the help of a professional psychiatrist to alleviate anxiety and fear. In addition, activities that can help reduce the anxiety levels in geriatric individuals, such as socialization, physical activity and meditation, may prove to be of importance.

Ethics approval and consent to participate

The study was approved by the Ethics Committee at the Faculty of Dentistry of Ataturk University, Erzurum, Turkey (approval No. 2022/38). Informed consent forms were signed by all the 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.

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Original paper

Upper airway dimensions and the skeletal parameters in orthodontic patients who developed moderate–severe COVID-19 symptoms during the pandemic

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Abstract

Background. Large airway dimensions are associated with a rapid decline in the lung function and a higher risk of hospitalization. Therefore, the airway dimensions of healthy subjects who tested positive for coronavirus disease 2019 (COVID-19) may be associated with the severity of COVID-19 symptoms.

Objectives. The objectives of this study were to measure the upper airway dimensions and the craniofacial skeletal parameters in patients who tested positive for COVID-19, to compare the upper airway dimensions and the craniofacial skeletal parameters between patients who developed no/mild symptoms and those with moderate–severe COVID-19 symptoms, and to assess any association of the skeletal relationships (anteroposterior (AP) and vertical) and the upper airway dimensions with the severity of COVID-19 symptoms in adult subjects.

Material and methods. A total of 204 orthodontic patients who tested positive for COVID-19 were evaluated. Of these, only 137 met the inclusion criteria. The sample was further subdivided into 2 groups based on the severity of symptoms: cases (moderate–severe symptoms; $n = 56$); and controls (asymptomatic/mild symptoms; $n = 81$). The upper airway dimensions and the skeletal parameters were measured on lateral cephalograms. The nonparametric Mann–Whitney *U* test was used to detect differences between the cases and the controls. Binary logistic regression analysis was used to evaluate the association between the studied variables and the severity of symptoms.

Results. The cases had a reduced lower face height (LFH) and a reduced perpendicular distance from the hyoid bone to the line connecting the anteroinferior limit of the 3rd cervical vertebra (C3) and the retrognathion point (RGN) (HH1) as compared to the controls. Regression analysis revealed a significant association of LFH ($p = 0.013$), the vertical airway length (VAL) ($p = 0.002$) and HH1 ($p = 0.021$) with the severity of COVID-19 symptoms.

Conclusions. The types of malocclusion were similar in the cases and the controls. Patients with reduced LFH and VAL, and a superiorly positioned hyoid bone in relation to the mandible developed more severe COVID-19 symptoms.

Keywords: symptoms, severity, skeletal, upper airway, COVID-19

Introduction

In late 2019, a new virus that caused respiratory infections emerged in Wuhan, China. Initially, the International Committee on Taxonomy of Viruses called it 2019 novel coronavirus (2019-nCoV), but later referred to it as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] The disease caused by the virus was named coronavirus disease 2019 (COVID-19). The virus is characterized by a rapid rate of spread, causing infection in many populations. Accordingly, the World Health Organization (WHO) declared the outbreak of a pandemic of international concern.[2] After 3 years of the pandemic, when it seemed that COVID-19 was moving toward the endemic status, global infections are rising again. Moreover, the reported number of cases is underestimated because of increasing unreported at-home testing.[3] Therefore, it is clear that SARS-CoV-2 will not be totally eliminated, which means continuing to adapt to life with COVID-19.[3]

The majority of patients with COVID-19 are asymptomatic or present with mild–moderate symptoms, including fever, generalized fatigue, vomiting, coughing, sneezing, and pneumonia.[4] However, a minority develop severe conditions and complications (acute respiratory distress syndrome, septic shock and multiple-organ failure in some patients). Unfortunately, in some cases, death is inevitable, with an estimated mortality rate between 1.4% and 4.3%.[5]

Worldwide, the gold standard test for the diagnosis of COVID-19 is real-time reverse transcription polymerase chain reaction (rRT-PCR).[6] However, other tests have been used with varying degrees of accuracy, such as viral detection in sputum, tracheal aspirate, bronchoalveolar lavage, and serological assays for immunoglobulins.[6] Saliva testing could serve as another tool for a quick and inexpensive diagnosis of COVID-19.[7] Additionally, although less precise in comparison with other tests, imaging techniques have been adopted in certain clinical situations.

The transmission of COVID-19 occurs through air droplets and contaminated surfaces. Therefore, preventive measures, such as the use of masks, hand washing and social distancing, have been in place since the start of the outbreak. However, the gold standard for prevention is vaccination.[8]

It has been found that although COVID-19 vaccines have proven efficacy in preventing severe illness and death among those exposed to the virus, a small percentage of people will still contract SARS-CoV-2.[9] In fact, the reported COVID-19 hospitalization rates were 4.1 times higher among 12–34-year-old individuals in the unvaccinated population than in those who had completed their primary series of the vaccine.[10]

Many risk factors have been linked to a greater risk of developing severe adverse outcomes. Patients at higher risk include older adults, those with respiratory and cardiovascular diseases, obese patients, and those with hypertension or diabetes mellitus.[11] Sleep disorders, including obstructive sleep apnea, have also been linked to severe consequences of COVID-19.[12] Furthermore, it has been reported that genetic factors might play a role in the expression of COVID-19 symptoms, although this issue is not fully understood. When compared to mild cases, patients with severe symptoms can exhibit significantly higher numbers of variants in coding and noncoding regions. Blood type has also been reported to influence the development of COVID-19 infection and its complications. The Rh(D)-positive blood type has been found more often in deaths due to COVID-19 infection. Also, different genomes have been linked to the severity of COVID-19.[13]

Oral manifestations of COVID-19 include dysgeusia (taste disorders), oral pain, blisters, desquamative gingivitis, and ulceration, especially of the tongue.[14] Although oral manifestations have been reported in about 25% of COVID-19 cases with severe complications, these manifestations usually occur as a result of an impaired immune system and/or susceptible oral mucosa.[15]

Inflammation can cause airway narrowing. As a result, the flow rate in the narrowed segment is increased. This enhances the tendency of the airway to collapse inward.[16]

Several methods to evaluate the upper airway dimensions have been reported, such as nasal resistance and airflow tests, nasoendoscopy, polysomnography, cone-beam computed tomography (CBCT), and lateral cephalograms (LCs).[17] The latter have been used extensively to investigate the airway dimensions.[18] Previous studies indicate that LCs are reliable in the assessment of the airway dimensions,[19] and that the commonly used landmarks to measure the airway dimensions can be reliably identified.[20–22]

Skeletal anteroposterior (AP) and vertical relationships may correlate with the upper airway dimensions or the total airway volume in healthy patients with no respiratory diseases or pathologies. It has been suggested that in some types of malocclusion, particularly Class I and Class II with increased vertical growth patterns, the upper airway tends to be narrower.[20] As a result, in addition to treating occlusal problems, the aim of several orthodontic treatment modalities is to prevent the reduction of the nasopharyngeal dimensions, or even help increase them; these modalities include mandibular advancement appliances, mandibular or maxillary surgeries, and rapid maxillary expansion. Oelsner et al. reported that large airway dimensions were associated with a rapid decline in the lung function and a higher risk of hospitalization and death.[21] Therefore, it would be worthwhile to investigate whether the airway dimensions of healthy subjects (with no comorbidities) who tested positive for COVID-19 are associated with the severity of COVID-19 symptoms. If such an association exists, this would highlight the importance of airway dimension assessment during orthodontic treatment planning.

The objectives of this study were to measure the upper airway dimensions and the craniofacial skeletal parameters in patients who tested positive for COVID-19, to compare the upper airway dimensions and the craniofacial skeletal parameters between patients who developed no/mild symptoms and those with moderate–severe COVID-19 symptoms, and to assess any association of the skeletal relationships (AP and vertical) and the upper airway dimensions with the severity of COVID-19 symptoms in adult subjects.

Material and methods

The study was conducted in accordance with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement.

Ethical approval for the study was obtained from the Institutional Review Board at Hamad Medical Corporation, Doha, Qatar (No. MRC-01-21-238).

Study design

This case–control study was carried out at Hamad Dental Center/Hamad Medical Corporation in the State of Qatar.

Participants and setting

Cases were patients who experienced moderate–severe COVID-19 symptoms that necessitated hospitalization and special care due to shortness of breath or severe complications (the need for supportive care, assisted ventilation or intensive care unit admission). Controls were COVID-19 patients with no/mild symptoms, for whom special care or hospital admission were not required. This was based on the emergency department decision that was recorded in the national electronic health record system, which is the only electronic health record system in the country (Cerner®, Kansas City, USA); in the system, each patient has a unique hospital identification number for both their medical and dental records.

The records of all orthodontic patients who tested positive for COVID-19 at any time between March 2020 and March 2021 were retrieved from the medical health record system of Hamad Medical Corporation in the State of Qatar. None of the included subjects was vaccinated while the study was being conducted. Eligible patients who met the inclusion criteria were included. The inclusion criteria were as follows:

- patients who tested positive for COVID-19;
- adult patients aged ≥18 years (the airway dimensions remain almost stable after turning 13 years of age)[17];
- no previous orthodontic treatment (some orthodontic procedures can affect the airway dimensions);
- no medical history of pharyngeal pathology and/or nasal obstruction, adenoidectomy, or tonsillectomy – it was determined based on the electronic health record system;
- non-pregnant, non-smokers, and no associated comorbidities, such as respiratory and cardiovascular diseases, hypertension, and diabetes mellitus;
- no missing posterior teeth, except for the third molars, which could affect the vertical dimension, since the posterior teeth act as a guide for the vertical jaw relationship (if there are no posterior teeth, this vertical guide is lost and, accordingly, the mandible overcloses, resulting in a reduction of the tongue space and more forward positioning of the mandible, which may affect the airway dimensions);
- LCs of sufficient quality available in the electronic medical records.

All LCs were taken in centric occlusion, using an OrthoSlice 1000C X-ray cephalogram machine (Trophy Radiologie, Marne-la-Vallée, France), with a cephalostat at 64 KVp, 16 mA and 0.64 s of exposure, according to the manufacturer's instructions.

The LCs of the included patients were printed out using the Xelis Dental 1.0, Dental 3D INFINITT PACS® software (INFINITT, Phillipsburg, USA). The LCs were hand-traced using acetate tracing paper. The tracing and landmark identification on the LCs were performed by one operator (T.M.A.). The measurement and assessment methods have been used previously.[17] The magnification of all radiographs was adjusted using a calibration marker.

The measurement of the upper airway dimensions was based on the identification of specific landmarks on the LC (Fig. 1). The definitions of the different points and measurements used are shown in Table 1.

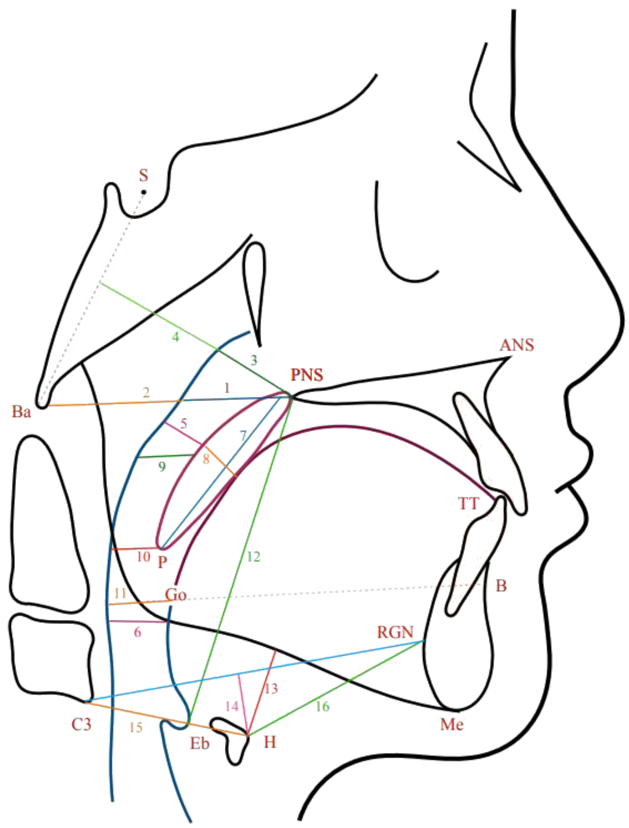

Fig. 1. Cephalometric skeletal base relationship, points and measurements used in the study to assess the upper airway dimensions (as defined in Table 1)

Table 1. Definitions of the airway points and measurements used in the study, with reference to Fig. 1

Cephalometric points		
Point	Description	
A	point A	
ANS	anterior nasal spine	
B	point B	
Ba	basion	
C3	anteroinferior limit of the 3rd cervical vertebra	
Eb	base of epiglottis	
Go	gonion	
H	hyoidale	
Me	menton	
P	tip of the soft palate	
PNS	posterior nasal spine	
RGN	retrognathion	
S	midpoint of the sella turcica	
TT	tongue tip	
Airway dimension measurements (in millimeters)		
No. in Fig. 1	Measurement	Description
1	PNS–AD1	lower airway thickness; distance between PNS and the nearest adenoid tissue measured through the PNS–Ba line (AD1)
2	AD1–Ba	lower adenoid thickness; defined as the soft-tissue thickness at the posterior nasopharynx wall through the PNS–Ba line
3	PNS-AD2	upper airway thickness; distance between PNS and the nearest adenoid tissue measured through a perpendicular line to S–Ba from PNS (AD2)
4	AD2-H	upper adenoid thickness; defined as the soft-tissue thickness at the posterior nasopharynx wall through the PNS–H line (H – hormion, point located at the intersection between a perpendicular line to S–Ba from PNS and the cranial base)
5	McN Upp	McNamara's upper pharynx dimension (minimum distance between the upper soft palate and the nearest point on the posterior pharynx wall)
6	McN Low	McNamara's lower pharynx dimension (minimum distance between the point where the posterior tongue contour crosses the mandible and the nearest point on the posterior pharynx wall)
7	PNS–P	soft palate length from PNS to the lower tip of the uvula (P)
8	MPT	soft palate thickness (maximum thickness of the soft palate measured on the line perpendicular to the PNS–P line)
9	SPAS	superior posterior airway space (width of the airway behind the soft palate along the line parallel to the Go–B line)
10	MAS	middle airway space (width of the airway along the line parallel to the Go–B line through P)
11	IAS	inferior airway space (width of the airway along the Go–B line)
12	VAL	vertical airway length (distance between PNS and Eb)
13	H–MP	perpendicular distance from the hyoid bone to the mandibular plane
14	HH1	perpendicular distance from the hyoid bone to the line connecting C3 and RGN
15	H–C3	distance between the hyoid bone and C3
16	H–RGN	distance between the hyoid bone and RGN
Skeletal relationships		
Relationship	Description	
Anteroposterior (AP)	ANB – angle formed between point A, nasion and point B Class I: 2–4° Class II: >4° Class III: <2°	
Vertical	Max/Mand – maxillary/mandibular planes angle reduced: <22° average: 27 ±5° increased: >32°	

For the AP skeletal relationship, the ANB angle was used to classify malocclusion into Class I, II or III. Vertically, the skeletal relationship was classified based on the maxillary/mandibular planes angle (Max/Mand) into reduced, average or increased (Table 1).

Outcomes

The primary outcome was the level of COVID-19 severity in relation to the upper airway dimensions and the skeletal relationships. The severity was assigned as either asymptomatic or moderate–severe.

The secondary outcome was the association between the body mass index (BMI) and COVID-19 severity.

Sample size

Convenient sampling was adopted. This was due to the lack of studies evaluating the effect of the upper airway dimensions on the severity of COVID-19 symptoms. Using the G*power program, v. 3.1.9 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower), and assuming a large effect size difference of 0.6 between the groups, the power analysis yielded a total sample size estimate of 54 subjects per group at a conventional alpha (α) level (0.05) and desired power (1-β) of 0.85.

Bias

To minimize bias, the investigator who carried out the tracing and made measurements was blinded to the patient's identity and to whether the patient was a case or a control. Independent variables (covariates) were also extracted from the electronic medical records. These included age and gender (demographics), height, weight, and BMI, which is calculated as weight [kg] / height $[m]^2$. The BMI was categorized as either underweight/normal if <25 kg/m^2 or overweight/obese if ≥ 25 kg/m^2.

Error of the method

A total of 10% of the included LCs were randomly selected and reanalyzed after a 2-week interval. Dahlberg's formula was used to calculate the method error (Equation 1)[23]:

$$ME = \sqrt{\left(\sum d^2 / 2N\right)} \quad (1)$$

where:
ME – method error;
d – difference between the measurements; and
N – sample size.

The Dahlberg error ranged from 0.27 mm for AD1–Ba to 0.68 mm for AD2–H.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, v. 28.0 (IBM Corp., Armonk, USA). Descriptive statistics were calculated for all the measured variables for each group. The Shapiro–Wilk test was used to assess the normality of the distribution of the numeric data; it indicated that the data was not normally distributed. The nonparametric Mann–Whitney *U* test was used to detect differences between the cases and the controls. In addition, odds ratios (*OR*s) were presented using binary logistic regression analysis to evaluate the association between the skeletal parameters and the upper airway dimensions and the severity of COVID-19 symptoms (dichotomized to either asymptomatic/mild (coded 1) or moderate–severe (coded 2)). The level of statistical significance was set at $p < 0.05$.

Results

Sample characteristics

A total of 204 patients diagnosed with COVID-19 were identified from the electronic medical records in the orthodontic department. Of those, 67 were excluded due to poor-quality LCs or not meeting the inclusion criteria. Ultimately, a total of 137 patients were included in the study (51 males and 86 females). The sample was further subdivided into 2 groups (cases and controls) based on the severity of COVID-19 symptoms. There were 56 cases (moderate–severe symptoms) and 81 controls (asymptomatic/mild symptoms) (Table 2). The mean age of the cases was 21.66 ±4.32 years, and the mean age of the controls was 21.95 ±5.02 years.

There were no differences between the groups in terms of gender ($p = 0.200$). Females comprised 68% of the cases and 59% of the controls, while only 32% of the cases and 41% of the controls were males (Table 2).

The percentage of overweight/obese patients among the cases was 70%, while it was only 48% among the controls. Subjects with a lower BMI (<25 kg/m^2) were less likely to develop severe COVID-19 symptoms ($p = 0.010$) (Table 2).

The types of malocclusion in the cases and the controls are presented in Table 2. No significant differences were detected between the 2 groups ($p = 0.584$). The Class II skeletal pattern was most represented in the cases, whereas in the controls, Class I and Class II were evenly distributed (37%).

Vertically, the majority of cases and controls had a normal vertical pattern (59% and 57%, respectively). There were more subjects with a reduced vertical pattern in the case group, whereas in the control group, there were more subjects with an increased vertical pattern. However, these differences were not statistically significant ($p = 0.248$) (Table 2).

Table 2. Characteristics of the cases and the controls in the study (*N* = 137)

Characteristic		Cases (*n* = 56)	Controls (*n* = 81)	χ^2	*p*-value
Gender	M	18 (32.1)	33 (40.7)	1.05	0.200
	F	38 (67.9)	48 (59.3)		
BMI	underweight/normal	17 (30.4)	42 (51.9)	6.24	0.010*
	overweight/obese	39 (69.6)	39 (48.1)		
AP skeletal classification	Class I	16 (28.6)	30 (37.0)	1.08	0.584
	Class II	24 (42.9)	30 (37.0)		
	Class III	16 (28.6)	21 (25.9)		
Vertical skeletal classification	reduced Max/Mand	10 (17.9)	8 (9.9)	2.79	0.248
	average Max/Mand	33 (58.9)	46 (56.8)		
	increased Max/Mand	13 (23.2)	27 (33.3)		

Data presented as number (percentage) (*n* (%)). BMI – body mass index; M – male; F – female; * statistically significant ($p < 0.05$).

The mean and standard deviation (*M* ±*SD*) values, the Mann–Whitney *U* test statistics, and *p*-values for the skeletal measurements in both the cases and the controls are shown in Table 3. The lower facial height (LFH) was the only cephalometric measurement that showed a significant association with severe COVID-19 symptoms ($p < 0.05$). Subjects who presented with severe COVID-19 symptoms had a reduced LFH as compared to the controls. There were no significant differences in ANB, Max/Mand, the palatal length (ANS–PNS), or the mandibular length (Go–Pog) between the cases and the controls ($p > 0.05$).

The *M* ±*SD* values, the Mann–Whitney *U* test statistics, and *p*-values for the airway dimensions in both the cases and the controls are shown in Table 4. In subjects with severe COVID-19 symptoms, the perpendicular distance from the hyoid bone to the line connecting C3 and RGN (HH1) was lower than in the controls ($p < 0.05$).

The results of binary regression analysis conducted to investigate the association between the skeletal parameters and the upper airway dimensions and the severity of COVID-19 symptoms are shown in Table 5. The following variables revealed significant associations: LFH ($p = 0.013$); vertical airway length (VAL) ($p = 0.002$); and HH1 ($p = 0.021$). The odds of developing moderate–severe COVID-19 symptoms increased by 1.2 times if LFH and HH1 were reduced by 1 unit. Also, as VAL was reduced by 1 unit, the odds of developing moderate–severe COVID-19 symptoms increased by 23%.

Discussion

Since the outbreak, COVID-19 has contributed to a large number of deaths and economic losses in a relatively short time. To date, in the State of Qatar, more than 247,000 cases have been diagnosed as COVID-19-positive out of a population of less than 3 million; 685 deaths were recorded until November 2022.[24] It was found that although the majority of infected patients had no or mild symptoms, approx. 15–25% developed severe symptoms with a relevant impairment of the respiratory function, leading to hospitalization and assisted ventilation.[4]

The upper airway has a complex geometry, and is surrounded by muscles and mobile soft tissue structures that can alter the airway configuration. The main function of the upper airway is to regulate the inspired and expired airflow, along with heating and humidifying the inspired air.[25] The relative growth and size of these surrounding tissues determine the size of the pharyngeal space, which plays an important role in the respiratory function and resistance to airflow.[26]

Table 3. Statistics for the skeletal measurements in both the cases and the controls

Variable	Cases	Controls	Mann–Whitney *U* test statistics	*Z*	*p*-value
ANB [°]	3.41 ±3.47	3.17 ±3.60	2165	−0.45	0.651
Max/Mand [°]	28.71 ±7.19	30.26 ±5.48	1872	−1.73	0.083
ANS–PNS [mm]	48.10 ±5.46	49.33 ±7.34	1934	−1.47	0.143
Go–Pog [mm]	69.21 ±7.14	71.16 ±7.33	1908	−1.58	0.115
UFH [mm]	48.84 ±4.12	49.68 ±4.07	2065	−0.89	0.372
LFH [mm]	59.95 ±5.44	62.44 ±5.98	1754	−2.25	0.024*
FP [%]	55.08 ±2.27	55.65 ±2.05	1856	−1.80	0.072

Data presented as mean ± standard deviation (*M* ±*SD*). Pog –pogonion; UFH – upper facial height; LFH – lower facial height; FP – facial proportions; * statistically significant ($p < 0.05$).

Table 4. Statistics for the airway dimensions in both the cases and the controls

Variable	Cases		Controls		Mann–Whitney *U* test statistics	*Z*	*p*-value
	M ±*SD*	mean rank	*M* ±*SD*	mean rank			
PNS–AD1 [mm]	23.25 ±3.57	74.04	22.38 ±5.43	65.52	1986	−1.24	0.216
AD1–Ba [mm]	18.77 ±4.26	64.59	19.95 ±5.17	72.05	2021	−1.09	0.278
PNS–AD2 [mm]	18.88 ±4.08	72.65	18.25 ±5.52	66.48	2063	−0.90	0.369
AD2–H [mm]	10.66 ±3.22	63.01	12.30 ±5.53	73.14	1932	−1.47	0.140
McN Upp [mm]	10.73 ±3.18	72.12	10.56 ±3.51	66.85	2093	−0.77	0.443
McN Low [mm]	12.02 ±3.92	73.33	11.12 ±3.54	66.01	2025	−1.07	0.286
PNS–P [mm]	33.34 ±5.10	68.18	33.59 ±5.51	69.57	2222	−0.20	0.840
MPT [mm]	7.61 ±1.69	63.04	7.98 ±1.73	73.12	1934	−1.48	0.138
SPAS [mm]	10.68 ±3.28	72.30	10.58 ±4.15	66.72	2083	−0.81	0.416
MAS [mm]	9.96 ±3.26	69.72	10.00 ±2.99	68.50	2227	−0.18	0.858
IAS [mm]	12.02 ±3.86	72.46	11.30 ±3.52	66.61	2074	−0.85	0.395
VAL [mm]	58.66 ±8.37	68.71	58.75 ±7.28	69.20	2251	−0.07	0.942
H–MP [mm]	13.16 ±4.23	63.65	14.17 ±4.49	72.70	1968	−1.32	0.188
HH1 [mm]	3.77 ±3.64	60.67	5.19 ±4.24	74.76	1801	−2.05	0.040*
H–C3 [mm]	32.81 ±5.40)	71.13	33.30 ±5.01	67.52	2148	−0.52	0.600
H–RGN [mm]	36.61 ±6.56	65.39	37.44 ±6.48	71.49	2066	−0.89	0.376

* statistically significant ($p < 0.05$).

Table 5. Results of binary regression analysis with the severity of symptom as the independent variable

Variable	β	*SE*	Wald statistics	*p*-value	Exp (β)	95% *CI* for Exp (β)
Gender	−0.250	0.55	0.199	0.656	0.780	0.27–2.30
Age	−0.002	0.05	0.003	0.957	0.998	0.91–1.09
BMI	0.060	0.05	1.233	0.267	1.060	0.96–1.17
ANB	0.010	0.08	0.014	0.905	1.010	0.86–1.18
Max/Mand	0.004	0.06	0.006	0.939	1.000	0.90–1.12
UFH	−0.070	0.07	0.861	0.353	0.930	0.81–1.08
LFH	−0.200	0.08	6.202	0.013*	0.820	0.70–0.96
PNS–AD1	−0.050	0.09	0.257	0.612	0.950	0.79–1.15
AD1–Ba	−0.090	0.07	1.618	0.203	0.920	0.80–1.05
PNS–AD2	0.030	0.08	0.189	0.664	1.030	0.89–1.20
AD2–H	−0.020	0.07	0.103	0.748	0.980	0.85–1.12
McN Upp	0.040	0.15	0.089	0.766	1.050	0.78–1.40
McN Low	0.240	0.22	1.272	0.259	1.280	0.84–1.95
PNS–P	−0.010	0.06	0.013	0.908	0.990	0.88–1.12
MPT	−0.07	0.14	0.247	0.619	0.930	0.71–1.23
SPAS	−0.080	0.14	0.387	0.534	0.920	0.71–1.20
MAS	−0.160	0.13	1.513	0.219	0.860	0.67–1.10
IAS	0.010	0.20	0.002	0.963	1.010	0.68–1.49
VAL	0.210	0.07	9.988	0.002**	1.230	1.08–1.40
H–MP	−0.120	0.08	2.205	0.138	0.890	0.77–1.04
HH1	−0.170	0.07	5.365	0.021*	0.840	0.73–0.97
H–C3	−0.100	0.07	1.760	0.184	1.100	0.96–1.26
H–RGN	−0.110	0.06	3.765	0.502	0.900	0.81–1.00

SE – standard error; *CI* – confidence interval; * statistically significant ($p < 0.05$); ** statistically significant ($p < 0.01$).
Cox–Snell R^2: 0.259. Hosmer–Lemeshow test: 6.69; $p = 0.570$.

This study investigated whether the upper airway dimensions and the skeletal relationships in healthy young adults (with no comorbidities) were associated with the severity of COVID-19 symptoms. If such an association exists, it would substantiate the importance of treating orthodontic problems that are associated with narrow airway dimensions as soon as they are identified. This study is considered the first to examine this association.

All age groups can be infected with COVID-19; however, the disease most commonly affects middle-aged and older adults. It was found that 80% of hospitalizations were in adults over 65 years of age, and that these patients were at a 23-fold greater risk of death than those aged less than 65 years.[27] In the present study, age ranged from 15.00 to 22.58 years, as all the included subjects in both groups were orthodontic patients from a young-age group with LCs in their medical records; this may be considered a limitation of this study.

All the included subjects were healthy and had no complaints of any systemic diseases, as comorbidities, such as hypertension, diabetes, obesity, and cardiovascular and respiratory diseases, are strongly associated with severe outcomes in COVID-19.[11]

In this study, gender was not associated with the severity of COVID-19 symptoms. This is in contrast to a report by Jin et al., who found that although the prevalence of COVID-19 was the same in males and females, males were at higher risk of developing severe outcomes and death, regardless of age.[28] It was also found in a meta-analysis conducted by Peckham et al. that male patients tended to develop more severe outcomes than females, with a significantly higher number of intensive care unit admissions and deaths.[29] The number of females in our study was almost ⅔ of the sample as compared to ⅓ being male patients. This difference may have affected our results.

In this study, BMI was significantly associated with the severity of COVID-19 complications. The overweight/obese patients were 2.7 times more likely to develop severe complications than the underweight/normal subjects. This finding agrees with that reported in previous studies, indicating an increased risk of severe COVID-19 complications among patients of higher weight. In their study, Gao et al. reported that a BMI of more than 23 kg/m^2 was associated with a linear increase in the risk of severe COVID-19, leading to hospital admission and death.[30] Another study from Korea by Kang and Kong found a relationship between BMI and fatal illness. They reported that a BMI of <18.5 kg/m^2 and a BMI of ≥ 25 kg/m^2 were associated with a higher risk of fatal illness.[31]

In the present study, the majority of subjects with moderate–severe COVID-19 symptoms presented with Class II malocclusion and a normal vertical pattern, whereas the majority of subjects who developed no or mild symptoms had Class I and Class II malocclusion and a normal vertical pattern. This is compatible with a study by Silva et al., who stated that Class II patients with smaller mandibles tended to have reduced sagittal airway dimensions.[32] Moreover, Kirjavainen and Kirjavainen found that Class II malocclusion in a group of healthy patients with no respiratory disorders was related to narrower oro- and hypopharyngeal spaces as compared to controls with a Class I molar relationship.[33] Kim et al. reported that a constricted nasopharyngeal airway was associated with retruded mandible and maxilla.[34] On the other hand, Chokotiya et al. found that the airway dimensions were not significantly different between Class I, II and III skeletal relationships.[35] However, in the present study, regression analysis revealed no association between the skeletal relationships (AP and vertical) and the severity of COVID-19 symptoms. The only cephalometric variable that showed an association with the severity of COVID-19 symptoms was LFH. A reduced LFH in the case subjects cannot be taken as an indicator of a vertical growth pattern, as other vertical variables (Max/Mand and facial proportions (FP)) were within the normal range.

A reduced LFH indicates a reduced distance between the palatal and mandibular planes, which in turn points to reduced room to accommodate the tongue. In such cases, the tongue and its associated soft tissues would be forced posteriorly, resulting in the narrowing of the sagittal airway dimensions. When the sagittal airway dimensions decrease, the vertical airway dimensions tend to increase as a response, which has been found to be associated with an accelerated decline in the lung function and an increased risk of hospitalization and death.[21] Of note, it is relevant to mention that several studies have investigated the effect of orthodontic treatment on the upper airway dimensions. While the majority of studies found no significant clinical effect after treatment,[17] some studies found a significant increase in the upper airway dimensions, in particular with rapid maxillary expansion appliances.[36]

In this study, subjects who developed moderate–severe symptoms had a reduced VAL and a superiorly positioned hyoid bone. It has been shown that the hyoid bone position differs in each type of malocclusion. It is positioned inferiorly in Class I and Class II malocclusion,[37,38] and superiorly in Class III malocclusion.[39]

In the present study, the hyoid bone was found to be more superior to the mandible in the cases as compared to the controls. It is due to the fact that the majority of controls had Class I and II malocclusion, which is consistent with what has been previously reported.[37,38] The majority of cases, however, had Class II malocclusion, but the hyoid bone was still located superior to the mandible; this can be explained by the fact that although the dominant malocclusion type was Class II, the associated LFH and VAL were reduced, which means that there was less space for the tongue, resulting in the stretching of the associated soft tissues which are connected to the epiglottis, thereby elevating the hyoid bone more superiorly.

Limitations

One limitation of this study is its high female/male ratio. Another one is a convenient sample, in which only orthodontic patients diagnosed with COVID-19 who had good-quality pretreatment LCs were included. Moreover, the study is a retrospective case–control study, in which the classification of cases vs. controls was based on symptoms and the treatment already provided to the patient. Finally, it is a single-center study, so the generalization of the results may not be possible.

Conclusions

The types of malocclusion were similar in the case and control subjects. COVID-19 patients with moderate–severe airway complications tended to have reduced LFH and VAL, and a superiorly positioned hyoid bone in relation to the mandible. COVID-19 patients with BMI ≥ 25 kg/m^2 were more likely to develop moderate–severe airway complications.

Ethics approval and consent to participate

Ethical approval for the study was obtained from the Institutional Review Board at Hamad Medical Corporation, Doha, Qatar (No. MRC-01-21-238).

Data availability

All data generated and/or analyzed during this study is included in this published article.

Consent for publication

Not applicable.

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Original paper

Analyzing the psychological effects of the COVID-19 pandemic on Turkish dental professionals

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Abstract

Background. Due to the working conditions, while performing dental procedures, dental professionals may experience a sense fear and anxiety about coronavirus disease 2019 (COVID-19).

Objectives. The aim of this study was to assess the levels of fear and anxiety about COVID-19 among dental professionals by using the Turkish version of the Fear of COVID-19 Scale (FCV-19S) and the Coronavirus Anxiety Scale (CAS), and to explore the risk factors associated with the intensity of fear and anxiety.

Material and methods. This cross-sectional study was conducted between October 16 and October 23, 2020, during the normalization process, by sending an online survey to 813 dental professionals working in public and university hospitals in Turkey. The questionnaire contained questions about socio-demographic characteristics as well as epidemic-related questions. The levels of fear and anxiety were assessed by means of FCV-19S and CAS, respectively.

Results. The sample's mean scores were 18.48 ±5.47 for FCV-19S and 2.17 ±3.08 for CAS. Female participants expressed higher levels of fear of COVID-19 than male participants ($p < 0.05$).

Conclusions. The fear and anxiety levels in dentists during the COVID-19 pandemic were found to be high.

Keywords: Turkey, dental professionals, COVID-19, Fear of COVID-19 Scale, Coronavirus Anxiety Scale

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Introduction

In December 2019, multiple cases of pneumonia of unknown etiology were identified in Wuhan, China.[1] The World Health Organization (WHO) named it novel coronavirus disease 2019 (COVID-19) and declared the outbreak of a pandemic on March 11, 2020.[2] The first case of COVID-19 in Turkey was announced on March 11, 2020. Since then, to control COVID-19, the Turkish government implemented precautions, such as social distancing, self-quarantine, travel restrictions, the postponement of scientific, cultural and similar activities, the transition of all educational institutions to the online education system, and the closure of restaurants, museums, movie theaters, swimming pools, sports halls, and hairdressers. Curfew was declared for people over 65 years of age and those with chronic illnesses, defined as being at high risk of contracting the disease, and young people under 20. Moreover, the Turkish Ministry of Health decided to postpone non-urgent dental practices in dental clinics at public and university hospitals.[3] The Turkish Ministry of Health established filiation teams comprised of healthcare professionals (doctors, nurses, dentists) to isolate COVID-19-positive cases from the individuals suspected to be infected. The role of filiation teams was to follow up cases, visit households and collect samples for tests.[4] Turkey began the normalization process on June 1, 2020. Domestic and international travel restrictions were canceled, and kindergartens, restaurants, museums, sports centers, hairdressers, and shopping centers were reopened.[5] Prior to the normalization process, 164,769 cases of COVID-19 were diagnosed and 4,563 deaths occurred in Turkey.[3]

Given the rapid transmission of the disease and the working conditions of dental professionals (exposure to aerosols, saliva, blood, and contaminated body fluids while performing dental procedures), dentists are at extremely high risk of contracting the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes COVID-19.[6] The virus can be detected in infected patients' saliva, which is why saliva is taken into consideration in the diagnosis of COVID-19.[7,8] COVID-19 can cause ulcerations, desquamative gingivitis, xerostomia, mucositis, and stomatitis in the oral cavity.[9] During the COVID-19 pandemic, the number of patients attended by dentists decreased as compared to the pre-pandemic time.[10] In a study conducted among patients who visited Karabük Oral and Dental Health Training and Research Hospital in Turkey, it was observed that the average number of patients per month in the 1-year period before the pandemic was approx. 3.5 times greater as compared to the 1-year period after the COVID-19 pandemic.[11] The majority of dental professionals (80%) provided only emergency treatment,[10] while 5.1–16.9% postponed all dental procedures[10,12] and 13.8% closed their dental clinics.[10] During the pandemic, patients would go to the dentist in case of dental emergency, mostly because of pain (36.6%) and a fractured tooth (36.1%).[13] Although dentists do take extra personal protective measures while performing dental procedures, studies show that 16–24% of dentists working in Turkey have been infected with SARS-CoV-2.[14–16] Due to the cross-contamination caused by aerosols, dental professionals are anxious about being infected and transmitting the infection to their families and friends, which may affect their behavior and make them experience a sense of fear and anxiety.[17] Research has shown that 2.6% of dentists,[18] 5.1% of healthcare professionals[19] and 3.5% of medical students[20] received psychological support during the pandemic.

During the COVID-19 pandemic, studies evaluating individuals' psychological stress and anxiety used various validated scales, including the Generalized Anxiety Disorder 7-item (GAD-7) scale, the Hospital Anxiety and Depression Scale (HADS), the Depression, Anxiety and Stress Scale-21 (DASS-21), the Beck Depression Inventory (BDI), and the Hamilton Depression Rating Scale (HDRS).[21–25] In order to investigate the impact of the COVID-19 pandemic on mental health, psychometric assessment tools, including the Fear of COVID-19 Scale (FCV-19S)[22] and the Coronavirus Anxiety Scale (CAS),[26] have recently been developed. These scales have been translated into different languages to explore their psychometric properties and for validation,[24,27–31] and have been found to be consistent and reliable. The psychological impact of the COVID-19 pandemic on general populations, patients and students has been reported in previous studies,[23,32] while little attention has been given to the psychological distress of dental practitioners.[17,33] However, during the COVID-19 pandemic, knowing factors that affect dentists' fear and anxiety levels is important for the implementation of measures to protect dental professionals against the risk associated with their working conditions.[24] Also, socio-demographic characteristics, such as age, gender, personal and family history of illness, marital status, workplace, and work hours per week, can affect students' and physicians' level of fear of COVID-19.[34] Therefore, it is important to investigate the effect of socio-demographic factors on the level of fear of COVID-19.

To our knowledge, no study has yet psychometrically measured dental professionals' psychological responses to the pandemic in Turkey, using scales specific for COVID-19. Thus, there is an unmet need to understand the psychological impact of the COVID-19 pandemic on Turkish dental professionals. The aim of the present study was to assess the level of fear and anxiety about COVID-19 among dentists by using the Turkish version of FCV-19S and CAS, and to explore the factors associated with the intensity of fear and anxiety. The hypothesis was that the levels of fear and anxiety would be high in dentists due to a high risk of disease transmission.

Material and methods

This study was approved by the Research Ethics Committee at the Faculty of Medicine of the Suleyman Demirel University, Isparta, Turkey (2020/355). The study was carried out in accordance with the Declaration of Helsinki standards. Electronic informed consent was obtained from the participants prior to data collection.

Participants

This cross-sectional study was conducted using an online survey from October 16 to October 23, 2020, during the normalization process in Turkey. The inclusion criteria were as follows: (1) being professionally active in dentistry; and (2) accepting to contribute to the research. The exclusion criterion was leaving an incomplete form. The study population included both general dentists and specialists working in public and university hospitals. According to the data from the Turkish Dental Association, there were 15,597 active general dentists and specialists working in public and university hospitals at the time of conducting the survey. Considering this data, the sample size was calculated using a formula based on a 95% confidence level and a 2% margin of error with maximum heterogeneity. The recommended minimum sample size was 242. Before sending the survey to all participants, a pilot study was performed on 20 selected dentists who were not in the same age group. Based on the pilot study results, no changes to the survey were required. The individuals from the pilot study could not participate in the final trial. The contact information of dental professionals was obtained through e-mail, WhatsApp, Facebook, and Instagram. A total of 850 questionnaires were distributed among dental professionals by Google Forms (Alphabet Co., Mountain View, USA). A group of 813 participants completed in full the questionnaire forms.

Measures

The survey contained questions about socio-demographic characteristics as well as epidemic-related questions. The fear and anxiety levels of participants were assessed by means of FCV-19S and CAS, respectively.

The FCV-19S, with Cronbach's alpha (α) value of 0.82, consists of 7 items on a 5-point Likert scale, with scores ranging from 7 to 35.[22] In the study, both a bifactor model[22] and a two-factor model[27] were used. The validity and reliability of the Turkish version of FCV-19S were verified by Satici et al.[29]

The CAS consists of 5 items that assess the physiological anxiety among individuals with a 5-point Likert scale (rating from 0 to 4), with robust reliability (α = 0.93).[26] The psychometric properties and validity of the Turkish version of CAS were verified by Evren et al.[35]

Statistical analysis

The data was analyzed statistically using IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA). The participants' descriptive characteristics were examined. The association between gender and work setting was assessed with the χ^2 test. As the variables were not normally distributed, the Mann–Whitney *U* test and the Kruskal–Wallis test were used for the comparison of the groups. The Bonferroni–Dunn test results are shown in Latin letters in superscript. The level of statistical significance was set at a *p*-value of 0.05.

Cronbach's α, and the correlations between each item score and the total score were calculated. The correlation was considered as weak if $r < 0.30$, moderate if the *r*-value was in the range of 0.30–0.59, and strong if $r \geq 0.60$.[36] A *p*-value <0.001 was considered statistically significant for the correlations.

Results

Participant characteristics

Of the 813 respondents, more than half were females ($n = 553$; 68%). The majority (58.9%) were aged 23–30 years, followed by 27.3% aged 31–40 years. A total of 200 (24.6%) respondents had been working for more than 11 years, and 438 (53.9%) for less than 5 years. At the time of the survey, 39.4% reported that they treated an average of at least 11 patients a day, and 83.4% of them were working in public hospitals. The study population had an almost equal representation of single and married individuals. Ninety-three (11.4%) had systemic diseases. The characteristics of the respondents are shown in Table 1.

Only 52 (6.4%) dental professionals had been diagnosed as COVID-19-positive. In addition, 80.0% knew someone who had been infected with SARS-CoV-2, and 31.2% were members of filiation teams. While social media was the data tool most frequently used by dentists (70.4%), it was closely followed by television/news (65.2%). Uncertainty during the pandemic concerned more than half of the participants, and 61.4% were suspicious about being infected when they felt symptoms similar to those of COVID-19 (Table 2).

Results and factors associated with FCV-19S and CAS

The sample's mean scores were 18.48 ±5.47 with a range of 7–35 for FCV-19S, and 2.17 ±3.08 with a range of 0–20 for CAS. Females expressed higher levels of fear of COVID-19 than males (19.22 ±5.28 and 16.91 ±5.54, respectively) (Table 3). In addition, the results suggest a trend among young adults (age groups: 23–30 years

Table 1. Characteristics of the participants (*N* = 813)

Variable		Gender			Work setting			Total
		M	F	*p*-value	public hospital	university hospital	*p*-value	
Age [years]	23–30	120 (46.2)	359 (64.9)	<0.001*	208 (48.8)	271 (70.0)	<0.001*	479 (58.9)
	31–40	93 (35.8)	129 (23.3)		125 (29.3)	97 (25.1)		222 (27.3)
	41–54	36 (13.8)	58 (10.5)		81 (19.0)	13 (3.4)		94 (11.6)
	≥55	11 (4.2)	7 (1.3)		12 (2.8)	6 (1.6)		18 (2.2)
Professional experience [years]	0–5	107 (41.2)	331 (59.9)	<0.001*	193 (45.3)	245 (63.3)	<0.001*	438 (53.9)
	6–10	68 (26.2)	107 (19.3)		92 (21.6)	83 (21.4)		175 (21.5)
	11–14	27 (10.4)	42 (7.6)		39 (9.2)	30 (7.8)		69 (8.5)
	≥15	58 (22.3)	73 (13.2)		102 (23.9)	29 (7.5)		131 (16.1)
Average number of patients treated daily	1–3	24 (9.2)	64 (11.6)	0.001*	19 (4.5)	69 (17.8)	<0.001*	88 (10.8)
	4–6	60 (23.1)	165 (29.8)		55 (12.9)	170 (43.9)		225 (27.7)
	7–10	47 (18.1)	133 (24.1)		85 (20.0)	95 (24.5)		180 (22.1)
	≥11	129 (49.6)	191 (34.5)		267 (62.7)	53 (13.7)		320 (39.4)
Specialty	general dentistry	140 (53.8)	239 (43.2)	<0.001*	379 (100)	0 (0)	<0.001*	379 (46.6)
	restorative dentistry	12 (4.6)	32 (5.8)		4 (0.9)	40 (10.3)		44 (5.4)
	orthodontics	17 (6.5)	41 (7.4)		2 (0.5)	56 (14.5)		58 (7.1)
	endodontics	10 (3.8)	47 (8.5)		8 (1.9)	49 (12.7)		57 (7.0)
	oral surgery	23 (8.8)	17 (3.1)		6 (1.4)	34 (8.8)		40 (4.9)
	pediatric dentistry	14 (5.4)	87 (15.7)		10 (2.3)	91 (23.5)		101 (12.4)
	prosthodontics	18 (6.9)	29 (5.2)		9 (2.1)	38 (9.8)		47 (5.8)
	periodontology	16 (6.2)	38 (6.9)		6 (1.4)	48 (12.4)		54 (6.6)
	oral diagnostics and radiology	10 (3.8)	23 (4.2)		2 (0.5)	31 (8.0)		33 (4.1)
Marital status	single	92 (35.4)	310 (56.1)	<0.001*	160 (37.6)	242 (62.5)	<0.001*	402 (49.4)
	married	168 (64.6)	243 (43.9)		266 (62.4)	145 (37.5)		411 (50.6)
Systemic disease	yes	31 (11.9)	62 (11.2)	0.675	59 (13.8)	34 (8.8)	0.023	93 (11.4)
	no	229 (88.1)	491 (88.8)		367 (86.2)	353 (91.2)		720 (88.6)
Participant living	alone	59 (22.7)	186 (33.6)	<0.001*	87 (20.4)	158 (40.8)	<0.001*	245 (30.1)
	with a family	43 (16.5)	119 (21.5)		94 (22.1)	68 (17.6)		162 (19.9)
	with a partner	152 (58.5)	230 (41.6)		240 (56.3)	142 (36.7)		382 (47.0)
	with a friend/friends	6 (2.3)	18 (3.3)		5 (1.2)	19 (4.9)		24 (3.0)
Smoking/Alcohol consumption	smoking	108 (41.5)	97 (17.5)	<0.001*	116 (27.2)	89 (23.0)	0.165	205 (25.2)
	alcohol consumption	85 (32.7)	123 (22.2)	0.001*	90 (21.1)	118 (30.5)	0.002*	208 (25.6)
	none	121 (46.5)	386 (69.8)	<0.001*	265 (62.2)	242 (62.5)	0.924	507 (62.4)
Family member aged ≥65 years and/or with a chronic disease	≥65 years of age	142 (54.6)	288 (52.1)	0.274	236 (55.4)	194 (50.1)	0.076	430 (52.9)
	a chronic disease	168 (64.6)	386 (69.8)	0.081	289 (67.8)	265 (68.5)	0.453	554 (68.1)
	both	104 (40.0)	230 (41.6)	0.362	189 (44.4)	145 (37.5)	0.027*	334 (41.1)
Total		260 (32.0)	553 (68.0)	–	426 (52.4)	387 (47.6)	–	813 (100)

Data presented as number (percentage) (*n* (%)). M – male; F – female; * statistically significant (χ^2 test).

Table 2. Evaluation of the participants' characteristics according to the Fear of COVID-19 Scale (FCV-19S) and the Coronavirus Anxiety Scale (CAS)

Variable		*n* (%)	FCV-19S	*p*-value	FCV-19S psycho-logical	*p*-value	FCV-19S emotional	*p*-value	CAS	*p*-value
COVID-19 diagnosis[1]	yes	52 (6.4)	17.88 ±2.67	0.320	8.33 ±3.03	0.302	9.56 ±2.87	0.546	2.58 ±2.67	0.044*
	no	761 (93.6)	18.52 ±5.48		8.76 ±3.13		9.76 ±3.78		2.14 ±3.10	
If you have ever experienced symptoms similar to those of COVID-19, did you suspect that you had SARS-CoV-2?[2]	I felt similar symptoms and suspected COVID-19	499 (61.4)	19.25 ±5.44[a]	<0.001*	9.12 ±3.22[a]	<0.001*	10.12 ±2.65[a]	<0.001*	9.18 ±2.90[b]	<0.001*
	I felt similar symptoms, but didn't suspect COVID-19	78 (9.6)	17.41 ±5.38[b]		8.33 ±2.82[a]		9.08 ±2.85[b]		1.50 ±2.54[b]	
	I haven't felt any similar symptoms so far	236 (29.0)	17.21 ±5.29[b]		8.03 ±2.90[b]		9.18 ±2.90[b]		1.19 ±2.16[b]	
Did you know anyone infected with COVID-19?[1]	yes	650 (80.0)	18.47 ±5.42	0.859	8.74 ±3.11	0.835	9.73 ±2.76	0.682	2.21 ±3.14	0.278
	no	163 (20.0)	18.55 ±5.68		8.72 ±3.22		9.83 ±2.86		1.99 ±2.82	
If yes, who was it?[1]	1st degree relatives	155 (19.1)	18.68 ±5.64	0.722	8.94 ±3.29	0.403	9.74 ±2.77	0.968	2.57 ±3.51	0.087
	2nd and 3rd degree relatives	227 (27.9)	18.42 ±5.10	0.849	8.69 ±2.90	0.921	9.73 ±2.63	0.862	2.09 ±3.11	0.463
	friends	463 (56.9)	18.58 ±5.45	0.500	8.72 ±3.09	0.964	9.86 ±2.79	0.157	2.32 ±3.07	0.018*
	neighbors	204 (25.1)	19.08 ±5.72	0.096	9.13 ±3.33	0.062	9.95 ±2.86	0.143	2.68 ±3.48	0.002*
What is your source of information about COVID-19?[1]	social media	572 (70.4)	18.72 ±5.45	0.096	8.82 ±3.19	0.423	9.91 ±2.68	0.032*	2.21 ±3.01	0.116
	Ministry of Health/ WHO websites	472 (58.1)	18.86 ±5.66	0.024*	8.97 ±3.20	0.009*	9.89 ±2.88	0.059	2.43 ±3.37	0.054
	television/ news	530 (65.2)	18.79 ±5.45	0.028*	8.88 ±3.11	0.058	9.91 ±2.75	0.023*	2.23 ±3.04	0.195
	research articles	211 (26.0)	19.44 ±5.20	0.001*	9.14 ±2.94	0.006*	10.30 ±2.68	0.001*	2.19 ±3.04	0.940
	none	46 (5.7)	15.37 ±5.11	<0.001*	7.09 ±2.44	<0.001*	8.28 ±3.02	0.001*	1.04 ±2.13	0.002*
Have you taken a role in filiation teams?[1]	yes	254 (31.2)	18.04 ±5.69	0.046*	8.65 ±3.23	0.371	9.39 ±2.92	0.014*	2.54 ±3.49	0.180
	no	559 (68.8)	18.68 ±5.36		8.77 ±3.08		9.91 ±2.70		2.00 ±2.86	
How long have you been a member of a filiation team? [months][2]	0	559 (68.8)	18.68 ±5.36	0.056	8.77 ±3.08	0.240	9.91 ±2.70[a]	0.033*	2.00 ±2.86	0.642
	0–1	73 (9.0)	18.58 ±5.55		8.95 ±3.17		9.63 ±2.73[ab]		2.44 ±3.61	
	2–3	106 (13.0)	17.22 ±5.44		8.15 ±3.00		9.07 ±2.93[b]		2.35 ±2.92	
	4–5	34 (4.2)	17.68 ±5.40		8.59 ±3.06		9.09 ±3.10[ab]		2.71 ±3.94	
	≥6	41 (5.0)	19.49 ±6.57		9.46 ±3.89		10.02 ±3.05[a]		3.10 ±4.21	
Are your working and resting areas crowded during the day?[1]	yes	597 (73.4)	18.80 ±5.47	0.007*	8.88 ±3.14	0.027*	9.92 ±2.74	0.006*	2.22 ±3.02	0.069
	no	216 (26.6)	17.59 ±5.40		8.32 ±3.05		9.28 ±2.85		2.02 ±3.22	
Do you feel uncertainty about the COVID-19 pandemic[2]	yes	511 (62.9)	20.60 ±4.90[a]	<0.001*	9.77 ±3.05[a]	<0.001*	10.83 ±2.38[a]	<0.001*	2.85 ±3.45[a]	<0.001*
	sometimes	268 (33.0)	15.56 ±4.16[b]		7.23 ±2.36[b]		8.33 ±2.24[b]		1.15 ±1.87[b]	
	no	34 (4.2)	9.68 ±2.56[c]		4.97 ±1.36[c]		4.71 ±1.45[c]		0.00 ±0.00[c]	

Data presented as *n* (%) or as mean ± standard deviation (*M* ±*SD*). COVID-19 – coronavirus disease 2019; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; WHO – World Health Organization; different letters in superscript show differences in the mean rank; * statistically significant ([1] Mann–Whitney *U* test; [2] Kruskal–Wallis test).

Table 3. Analyzing the scales according to the gender and work setting of dental professionals

Scale	Item	Gender			Work setting			Total
		M	F	*p*-value	public hospital	university hospital	*p*-value	
FCV-19S	1. I am most afraid of COVID-19.	3.03 ±1.09	3.44 ±0.97	<0.001*	3.27 ±1.07	3.36 ±0.99	0.280	3.31 ±1.03
	2. It makes me uncomfortable to think about COVID-19.	3.18 ±1.18	3.76 ±0.97	<0.001*	3.56 ±1.11	3.59 ±1.04	0.850	3.57 ±1.08
	3. My hands become clammy when I think about COVID-19.	1.78 ±0.90	1.97 ±0.93	0.002*	1.95 ±0.97	1.87 ±0.88	0.348	1.91 ±0.93
	4. I am afraid of losing my life because of COVID-19.	2.76 ±1.25	2.91 ±1.17	0.065	2.79 ±1.21	2.94 ±1.18	0.084	2.86 ±1.20
	5. When watching news and stories about COVID-19 on social media, I become nervous or anxious.	2.74 ±1.15	3.38 ±1.01	<0.001*	3.17 ±1.09	3.19 ±1.11	0.588	3.18 ±1.10
	6. I cannot sleep because I'm worrying about getting COVID-19.	1.62 ±0.82	1.70 ±0.82	0.107	1.74 ±0.85	1.60 ±0.78	0.018*	1.67 ±0.82
	7. My heart races or palpitates when I think about getting COVID-19.	1.81 ±0.98	2.04 ±1.05	0.002*	2.02 ±1.06	1.91 ±1.00	0.147	1.97 ±1.03
	total FCV-19S score	16.91 ±5.54	19.22 ±5.28	<0.001*	18.50 ±5.71	18.46 ±5.21	0.798	18.48 ±5.47
	FCV-19S psychological score	7.94 ±3.11	9.10 ±3.07	<0.001*	8.88 ±3.24	8.57 ±2.99	0.301	8.73 ±3.13
	FCV-19S emotional score	8.97 ±2.96	10.12 ±2.62	<0.001*	9.62 ±2.89	9.89 ±2.66	0.208	9.75 ±2.78
	How often have you experienced the following problems over the last 2 weeks?							
CAS	1. I felt dizzy, lightheaded or faint when I read or listened to news about the coronavirus.	0.20 ±0.58	0.42 ±0.79	<0.001*	0.37 ±0.77	0.34 ±0.70	0.775	0.35 ±0.73
	2. I had trouble falling or staying asleep, because I was thinking about the coronavirus.	0.41 ±0.77	0.67 ±0.89	<0.001*	0.66 ±0.93	0.51 ±0.78	0.077	0.59 ±0.86
	3. I felt paralyzed or frozen when I thought about or was exposed to information about the coronavirus.	0.16 ±0.03	0.25 ±0.63	<0.001*	0.25 ±0.66	0.19 ±0.56	0.245	0.22 ±0.61
	4. I lost interest in eating when I thought about or was exposed to information about the coronavirus.	0.44 ±0.05	0.54 ±0.78	0.007*	0.58 ±0.87	0.44 ±0.68	0.073	0.51 ±0.79
	5. I felt nauseous or had stomach problems when I thought about or was exposed to information about the coronavirus.	0.32 ±0.75	0.57 ±0.89	<0.001*	0.59 ±0.94	0.39 ±0.75	0.003*	0.49 ±0.85
	total CAS score	1.54 ±2.81	2.46 ±3.15	<0.001*	2.44 ±0.36	1.87 ±2.70	0.003*	2.17 ±3.08

Data presented as *M* ±*SD*. * statistically significant (Mann–Whitney *U* test).

and 31–40 years) to have higher scores on FCV-19S and CAS than other participants, but still no statistically significant differences were observed ($p > 0.05$). Other socio-demographic characteristics of the participants, including their professional experience, the average number of patients treated daily, the marital status, the people the participants live with, and having a family member over 65 years of age and/or with a chronic disease, had no statistically significant effect on the FCV-19S and CAS scores ($p > 0.05$). In contrast, professional areas did affect the fear and anxiety levels ($p < 0.05$) (Table 4). Individuals who had had a COVID-19 diagnosis showed much lower FCV-19S scores as compared to those who had not, but this difference was not statistically significant ($p > 0.05$). The FCV-19S and CAS scores of the participants who stated that they were suspicious when they felt symptoms similar to those of COVID-19, and stated that they were worried about the uncertainty regarding COVID-19 were found to be statistically significantly higher in comparison with the scores of others ($p < 0.05$). Knowing an individual who had been diagnosed with COVID-19 had no effect on the FCV-19S and CAS scores ($p > 0.05$) (Table 2).

Psychometric properties

The Cronbach's α measure of internal consistency was 0.875 for FCV-19S and 0.852 for CAS, suggesting that both scales had sufficiently high reliability. The reliability of the scale was found to be very high for the corrected item–total correlations of all 7 items in FCV-19S (all r-values ≥0.70). The inter-item correlations ranged between 0.397 and 0.709 for FCV-19, and between 0.398 and 0.607 for CAS. The results of the correlation analysis showed that the FCV-19 scores were strongly positively correlated with the FCV-19 psychological ($r = 0.932$; $p < 0.001$) and emotional ($r = 0.930$; $p < 0.001$) response scores. The CAS scores were found to be moderately positively correlated with the FCV-19S scores ($r = 0.566$; $p < 0.001$). It was observed that the higher an individual's perceived stress level, the higher their anxiety level was (Table 5 and Table 6).

Table 4. Analyzing the scales according to the participants' characteristics

Variable		FCV-19S	*p*-value	FCV-19S psycho-logical	*p*-value	FCV-19S emotional	*p*-value	CAS	*p*-value
Age [years][2]	23–30	18.59 ±5.34	0.180	8.78 ±3.05	0.388	9.81 ±2.74	0.097	2.07 ±2.93	0.152
	31–40	18.73 ±5.73		8.81 ±3.29		9.93 ±2.80		2.47 ±3.24	
	41–54	17.82 ±5.36		8.53 ±3.13		9.29 ±2.77		2.16 ±2.49	
	≥55	16.00 ±5.70		7.67 ±3.12		8.33 ±3.24		1.06 ±2.01	
Professional experience [years][2]	0–5	18.63 ±5.42	0.439	8.82 ±3.14	0.630	9.81 ±2.74	0.237	2.07 ±2.92	0.317
	6–10	18.71 ±5.45		8.75 ±3.07		9.95 ±2.77		2.52 ±3.23	
	11–14	18.28 ±5.59		8.52 ±3.12		9.75 ±2.81		2.13 ±3.39	
	≥15	17.79 ±5.59		8.53 ±3.20		9.27 ±2.90		2.05 ±3.21	
Average number of patients treated daily[2]	1–3	17.81 ±4.69	0.613	8.32 ±2.64	0.708	9.49 ±2.48	0.476	1.99 ±2.51	0.779
	4–6	18.48 ±5.45		8.76 ±3.05		9.72 ±2.75		2.20 ±2.90	
	7–10	18.79 ±5.23		8.77 ±3.15		10.02 ±2.52		2.27 ±3.42	
	≥11	18.49 ±5.82		8.80 ±3.29		9.69 ±3.02		2.14 ±3.14	
Specialty[2]	general dentistry	18.51 ±5.82[abc]	0.019*	8.92 ±3.27[abc]	0.007*	9.59 ±2.97[ab]	0.026*	2.42 ±3.28[ab]	0.012*
	restorative dentistry	18.73 ±6.06[ab]		8.86 ±3.50[abc]		9.86 ±2.95[ab]		2.39 ±3.38[ab]	
	orthodontics	18.57 ±3.96[bc]		8.67 ±1.99[ab]		9.90 ±2.43[ab]		1.48 ±2.11[bc]	
	endodontics	19.60 ±5.42[a]		9.21 ±3.17[a]		10.39 ±2.52[a]		3.05 ±3.56[a]	
	oral surgery	17.00 ±4.11[bc]		7.35 ±2.49[d]		9.65 ±2.17[ab]		1.45 ±2.14[c]	
	pediatric dentistry	19.30 ±4.21[a]		8.93 ±2.54[a]		10.37 ±2.36[a]		1.70 ±2.72[c]	
	prosthodontics	17.21 ±5.57[bc]		7.92 ±3.32[cd]		9.30 ±2.88[ab]		1.62 ±3.27[c]	
	periodontology	16.83 ±6.44[c]		8.07 ±3.60[bcd]		8.76 ±3.10[b]		2.13 ±2.95[bc]	
	oral diagnostics and radiology	19.52 ±5.08[a]		8.94 ±3.30[ab]		10.58 ±2.21[a]		1.76 ±1.95[abc]	
Marital status[1]	single	18.56 ±5.43	0.498	8.76 ±3.11	0.676	9.80 ±2.74	0.626	2.20 ±3.13	0.951
	married	18.41 ±5.51		8.71 ±3.14		9.70 ±2.83		2.13 ±3.03	
Systemic disease[1]	yes	20.65 ±5.83	<0.001*	10.07 ±3.40	<0.001*	10.58 ±2.86	0.002*	3.23 ±4.11	0.008*
	no	18.20 ±5.36		8.56 ±3.05		9.64 ±2.76		2.03 ±2.89	
Participant living[1]	alone	18.51 ±5.42	0.836	8.75 ±3.09	0.630	9.76 ±2.70	0.984	2.01 ±2.98	0.191
	with a family	18.71 ±5.46		8.90 ±3.20		9.82 ±2.77		2.48 ±3.58	
	with a partner	18.35 ±5.47		8.62 ±3.09		9.73 ±2.82		2.07 ±2.86	
	with a friend/friends	18.75 ±6.32		9.29 ±3.67		9.46 ±3.18		3.21 ±3.60	
Smoking/Alcohol consumption[1]	smoking	17.81 ±5.46	0.038*	8.39 ±3.07	0.056	9.42 ±2.89	0.083	2.19 ±3.35	0.314
	alcohol consumption	18.25 ±5.57	0.427	8.62 ±3.27	0.352	9.63 ±2.76	0.463	2.56 ±3.25	0.013*
	none	18.64 ±5.55	0.206	8.82 ±3.16	0.251	9.82 ±2.81	0.284	2.06 ±2.97	0.335
Family member aged ≥65 years and/or with a chronic disease[1]	≥65 years of age	18.31 ±5.56	0.339	8.66 ±3.17	0.398	9.65 ±2.87	0.321	2.19 ±3.03	0.525
	a chronic disease	18.74 ±5.55	0.064	8.88 ±3.15	0.039*	9.85 ±2.84	0.092	2.32 ±3.18	0.036*
	both	18.39 ±5.66	0.594	8.71 ±3.21	0.617	9.68 ±2.92	0.695	2.22 ±3.08	0.622

Data presented as *M* ±*SD*. Different letters in superscript show differences in the mean rank; * statistically significant ([1] Mann–Whitney *U* test; [2] Kruskal–Wallis test).

Table 5. Factor analysis and the measures of internal consistency reliability of the Fear of COVID-19 Scale (FCV-19S)

Item	Descriptive statistics		Factor loading	Corrected item–total correlation *r*-value *p*-value	Inter-item correlations *r*-value *p*-value							Variance
	M ±*SD*	*Me*			1	2	3	4	5	6	7	
1. I am most afraid of COVID-19.	3.31 ±1.03	3	0.768	0.774 <0.001*	1.000	0.606 <0.001*	0.494 <0.001*	0.566 <0.001*	0.566 <0.001*	0.443 <0.001*	0.471 <0.001*	57.677
2. It makes me uncomfortable to think about COVID-19.	3.57 ±1.08	4	0.754	0.757 <0.001*	0.606 <0.001	1.000	0.432 <0.001*	0.496 <0.001*	0.648 <0.001*	0.397 <0.001*	0.440 <0.001*	13.197
3. My hands become clammy when I think about COVID-19.	1.91 ±0.93	2	0.745	0.732 <0.001*	0.494 <0.001*	0.432 <0.001*	1.000	0.509 <0.001*	0.466 <0.001*	0.562 <0.001*	0.570 <0.001*	7.807
4. I am afraid of losing my life because of COVID-19.	2.86 ±1.20	3	0.757	0.773 <0.001*	0.566 <0.001*	0.496 <0.001*	0.509 <0.001*	1.000	0.476 <0.001*	0.459 <0.001*	0.515 <0.001*	6.551
5. When watching news and stories about COVID-19 on social media, I become nervous or anxious.	3.18 ±1.10	3	0.766	0.771 <0.001*	0.556 <0.001*	0.648 <0.001*	0.466 <0.001*	0.476 <0.001*	1.000	0.416 <0.001*	0.507 <0.001*	5.797
6. I cannot sleep because I'm worrying about getting COVID-19.	1.67 ±0.82	2	0.738	0.706 <0.001*	0.443 <0.001*	0.397 <0.001*	0.562 <0.001*	0.459 <0.001*	0.416 <0.001*	1.000	0.709 <0.001*	4.840
7. My heart races or palpitates when I think about getting COVID-19.	1.97 ±1.03	2	0.787	0.772 <0.001*	0.471 <0.001*	0.440 <0.001*	0.570 <0.001*	0.515 0.507	0.507 <0.001*	0.709 <0.001*	1.000	4.131
Total FCV-19S	18.48 ±5.47	18	AVE	–	–	–	–	–	–	–	–	–
Cronbach's α	0.875		CR	–	–	–	–	–	–	–	–	–

Me – median; AVE – average variance extracted; CR – composite reliability; * statistically significant ($p < 0.001$).

Table 6. Factor analysis and the measures of internal consistency reliability of the Coronavirus Anxiety Scale (CAS)

Item	Descriptive statistics		Factor loading	Corrected item–total correlation *r*-value *p*-value	Inter-item correlations *r*-value *p*-value					Variance
	M ±*SD*	*Me*			1	2	3	4	5	
1. I felt dizzy, lightheaded or faint when I read or listened to news about the coronavirus.	0.35 ±0.73	0	0.779	0.665 <0.001*	1.000	0.530 <0.001*	0.550 <0.001*	0.398 <0.001*	0.480 <0.001*	63.488
2. I had trouble falling or staying asleep, because I was thinking about the coronavirus.	0.59 ±0.86	0	0.818	0.832 <0.001*	0.530 <0.001	1.000	0.466 <0.001*	0.536 <0.001*	0.555 <0.001*	11.876
3. I felt paralyzed or frozen when I thought about or was exposed to information about the coronavirus.	0.22 ±0.61	0	0.789	0.575 <0.001*	0.550 <0.001*	0.466 <0.001*	1.000	0.436 <0.001*	0.457 <0.001*	9.229
4. I lost interest in eating when I thought about or was exposed to information about the coronavirus.	0.49 ±0.85	0	0.798	0.773 <0.001*	0.398 <0.001*	0.536 <0.001*	0.436 <0.001*	1.000	0.607 <0.001*	8.289
5. I felt nauseous or had stomach problems when I thought about or was exposed to information about the coronavirus.	3.18 ±1.10	0	0.800	0.778 <0.001*	0.480 <0.001*	0.555 <0.001*	0.457 <0.001*	0.607 <0.001*	1.000	7.118
Total CAS	2.17 ±3.08	0	AVE	–	–	–	–	–	–	–
Cronbach's α	0.852		CR	–	–	–	–	–	–	–

* statistically significant ($p < 0.001$).

Discussion

The present study was conducted from October 16 to October 23, 2020, when the number of COVID-19 cases was increasing around the world during the 2nd wave of the pandemic. On October 16, 2020, when we started the survey, 343,955 people had tested positive for COVID-19 in Turkey; 1,812 of them were novel cases. Up to that day, the total number of deaths had been reported to be 9,153.[3] According to the WHO, as of October 16, 2020, more than 30 million confirmed cases and more than 1 million deaths worldwide had been reported.[2] At the end of the survey on October 23, 2020, there were 357,693 confirmed cases, 2,165 of them novel, and 9,658 people had died in Turkey.[3] However, this data does not reflect the true prevalence of COVID-19, since COVID-19 tests are performed on people with symptoms. Thus, efforts to diagnose the majority of those who have mild symptoms or are asymptomatic have failed.

Dental professionals are at the front line in the fight against the pandemic, and are vulnerable to debilitating fear and anxiety. Many published studies found high levels of psychological distress among dental professionals.[17,23,32] In order to understand the psychological effects of COVID-19, the fear and anxiety levels in individuals must be measured using psychometric tools. The first documented psychopathology-related tests for COVID-19 are FCV-19S and CAS.[21] The FCV-19S and CAS scores in our study were lower as compared to some other similar studies,[21,22,24,26,28,29,35,37] but higher than those reported in others.[27,30] The discrepancies among these studies may be caused by differences in culture, the age of participants, the study design, the population, and the time of data collection.

Female participants had higher FCV-19 and CAS scores than male participants in this study. This may be due to the imbalance in the sample's gender composition (the scarcity of male participants) or the gender differences in sensitivity to stress, which was also identified in recent studies.[23,27,28,31,38] Conversely, there are also studies showing that gender does not affect the fear levels.[22,33,39]

Although aging increases the risk of COVID-19 infection and the mortality rate,[40] our findings show that the FCV-19S scores were higher in the younger age groups (23–30 years and 31–40 years) than in older individuals; however, age was not significantly associated with the FCV-19S or CAS scores ($p > 0.05$). This result is consistent with those of previous studies with regard to age differences.[30,41] Older dental professionals with more professional experience are thought to have lower FCV-19S and CAS scores due to their better stress management, as they have experienced many similar situations throughout their lives. In a previous study, dentists who were older were more likely to think about retiring early and changing their career away from dentistry.[42] Consequently, there were conflicting results regarding differences in the levels of fear of COVID-19 between older and younger individuals.[43]

In this study group, the percentages of general dentists and specialists were similar. The levels of fear and anxiety differed according to professional areas. The lowest FCV-19S scores were observed in periodontists, followed by oral surgeons, prosthodontists, and orthodontists. However, differences in the number of male and female specialists working in their professional areas may have affected the scores. The long duration of treatment and thoughts of exposure to a greater viral load may have caused higher FCV-19S scores in pedodontists and endodontists. In addition, higher scores in oral diagnostics and radiology specialists may be due to large numbers of patients they attend and to a wide age range of patients. The age-related risk factors may influence the anxiety levels of radiologists due to the fact that this department includes patients from all age groups.

In this study, the number of patients treated daily had no effect on dentists' stress levels. However, reducing the number of patients treated daily and increasing the chair time between two patients can be helpful in preventing cross-infection, as there is time to disinfect contaminated areas.[44] While 70.4% of the dentists who treated ≥7 patients daily worked in public hospitals, no difference was found between institutions in terms of FCV-19S scores ($p > 0.05$). It is thought that continuing education while working in university hospitals may cause mental fatigue, and that treating more patients daily in public hospitals may increase physical fatigue in dentists. The fact that dentists working in public hospitals are more open to external stimuli may have contributed to their statistically significantly higher CAS scores ($p < 0.05$).

Although it is thought that being married and living with a family may influence dentists' stress levels due to their concern not only for themselves, but also for the health of the individuals with whom they live, in our study, which had an almost equal representation of single and married individuals, these factors had no effects on the FCV-19S and CAS scores. In a previous study, single people were found to have a higher level of fear of COVID-19 than married people.[45] The lack of social support may be associated with high levels of fear of COVID-19.

Given the important role of the body's immune system, people who have chronic medical conditions are at higher risk of becoming infected with SARS-CoV-2 and being hospitalized due to COVID-19.[33] Thus, it was not surprising to find higher FCV-19S and CAS scores in dentists who cope with systemic illnesses than in healthy participants in this study. There are also studies showing that systemic illnesses are associated with increased levels of distress.[25,31] Having asthma, diabetes, cardiovascular disease symptoms, and more than one disease increased experiencing mild to extremely severe levels of stress in comparison with healthy individuals.[46] The disruption of the healthcare systems during the pandemic may contribute to increased anxiety and depression scores. Among individuals diagnosed with COVID-19, the anxiety prevalence was reported to be 47% (34–61%).[23] In this study, since almost all of the dentists diagnosed with COVID-19 were under the age of 40, no statistically significant relationship was observed between a COVID-19 diagnosis and the FCV-19S scores. However, in people with a diagnosis of COVID-19, the news in various media informing of a possible increase in future health problems may cause higher CAS scores.[32] In our study, no effect of having a relative being diagnosed with COVID-19 on the FCV-19 and CAS scores was observed; however, in similar studies, the anxiety levels were found to be higher in people with at least one family member, relative or friend with a COVID-19 diagnosis.[25,31,32,37]

In our study, lower FCV-19S scores were observed in dentists who had a role in filiation teams. This result is thought to be due to the fact that dentists mainly worked in the field in the early stages of the COVID-19 pandemic, while most of them worked on the patient-tracking system during our survey period. However, it has been reported that healthcare workers can have increased stress levels due to their working outside the hospital conditions, to which they are accustomed.[47] Avoiding being in crowded areas, following the social distancing rules and minimizing social contact can slow the spread of SARS-CoV-2. However, 73.4% of the dentists participating in our study stated that they could not always apply these rules in their working and resting areas, and in this group, a thought of an increased risk of being infected with SARS-CoV-2 may have caused increased FCV-19S and CAS scores.

In this study, the fact that most of the dentists received news about COVID-19 via social media caused an increase in the levels of fear. The WHO has emphasized that people should minimize their exposure to news about COVID-19, obtain information only from reliable sources and only during certain hours, as spending a longer time watching COVID-19-related news is one of the important risk factors for distress.[23,48] This is in agreement with our finding that the FCV-19S and CAS scores were lower in dentists who did not follow information about COVID-19. The increasing anxiety related to the uncertainty regarding the COVID-19 pandemic among dental professionals is directly associated with misinformation about COVID-19, COVID-19's rapid transmission rate, and its morbidity and mortality rates. The FCV-19S and CAS scores increased significantly depending on the anxiety levels experienced due to uncertainty ($p < 0.05$).

During the pandemic, the WHO and the U.S. Centers for Disease Control and Prevention (CDC) prepared various recommendations to healthcare professionals on the management of stress, anxiety and uncertainty. In addition, the WHO offered psychological support to healthcare professionals in order to reduce the spread of COVID-19 among healthcare professionals. The CDC advice to healthcare professionals includes maintaining an adequate sleep and nutrition program, physical exercise, relaxation techniques, such as breathing exercises and meditation, taking a break from watching and reading news about the pandemic (especially on social media), and allocating time for hobbies outside of work.[49] However, due to the excessive workload of healthcare professionals, the application of these recommendations is quite limited.

Limitations and strengths

There are some limitations to the present study. First, since the fear and anxiety levels in the dental professionals participating in this study from the period prior to the pandemic are not known, an increase in these levels is unknown. Second, since this is a cross-sectional study, our findings reflect only a certain period of the pandemic. Third, during the data collection period, there were several online surveys related to COVID-19. Fourth, only scales related to COVID-19 were included, as it was thought that participants might be reluctant to participate in a longer questionnaire.

Notwithstanding these limitations, there are several strengths that distinguish our study from others. First, this study provides valuable data regarding the psychological effects of COVID-19 on Turkish dental professionals that were obtained using FCV-19S and CAS. Second, both the bifactor and two-factor models of FCV-19S were used. Third, the study participants were dentists who must have worked within severe restrictions in state institutions due to the pandemic.

Conclusions

In our study, which used scales specific for COVID-19, the fear and anxiety levels in dental professionals during the pandemic were found to be high. The continuing uncertainty, along with the prolonged period of the pandemic, cause these levels to increase. Healthcare professionals who are at risk, and therefore feel anxious, may find it difficult to provide adequate and quality service to all patients. It may be beneficial to enhance some programs, such as Mental Health Support System (RUHSAD), an online therapy outlet developed during the pandemic period, in order to provide psychological support to healthcare professionals.

In addition, providing the necessary conditions for healthcare workers as mandated by the state, complying with the rules required during the pandemic, and providing support to reduce the overload of healthcare professionals in the community would be beneficial in reducing the fear and anxiety levels.

Ethics approval and consent to participate

The ethical approval was provided by the Research Ethics Committee at the Faculty of Medicine of the Suleyman Demirel University, Isparta, Turkey (2020/355). All participants provided informed written consent prior to the investigations.

Data availability

All data analyzed during this study is included in this published article.

Consent for publication

Not applicable.

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Original paper

Salivary matrix metalloproteinase-9 (MMP-9) as a biomarker of periodontitis in pregnant patients with diabetes

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Abstract

Background. Pregnancy and diabetes increase the risk of developing pathological conditions in the periodontium. Salivary biomarkers, such as matrix metalloproteinase-9 (MMP-9), as well as antioxidants can be used as diagnostic indicators in monitoring periodontitis.

Objectives. The aim of the study was to assess the periodontal status of pregnant women with regard to the presence of diabetes. In addition, we aimed to assess antioxidant activity and the level of MMP-9 in saliva in order to establish the optimal noninvasive determinants of periodontitis.

Material and methods. The study included 104 pregnant women: 35 patients had gestational diabetes mellitus (GDM); 30 patients had type 1 diabetes (T1D); and 39 patients did not have diabetes (the control group). The physical examination included the assessment of the approximal plaque index (API), the gingival index (GI), bleeding on probing (BOP), the probing pocket depth (PPD), and clinical attachment loss (CAL). In the saliva study, MMP-9 concentration as well as the ferric reducing ability of plasma (FRAP), and the activity of superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPX) were measured.

Results. The pregnant patients with GDM and T1D had higher GI, BOP, PPD, and CAL scores than the control women ($p < 0.0001$, $p = 0.0040$, $p = 0.0100$, $p = 0.0030$, and $p < 0.0001$, $p < 0.0009$, $p < 0.0001$, $p < 0.0001$, respectively). The T1D patients had higher API scores as compared to the control women ($p = 0.0010$). The patients with periodontitis had higher salivary MMP-9 levels than the patients without periodontitis ($p = 0.0001$). The salivary antioxidant levels and activity were comparable among the study groups. The determinants of periodontitis ($p < 0.0001$) were MMP-9 concentration ($p = 0.0008$) and oral hygiene ($p = 0.0001$). The concentration of MMP-9 was also a useful determiner of the presence of periodontitis ($p < 0.0001$).

Conclusions. In the pregnant women with diabetes, we observed worse gingival conditions, deeper periodontal pockets and greater attachment loss in comparison with the women from the control group. The concentration of MMP-9 in saliva is a good predictor of periodontitis and might be a useful tool for diagnosing periodontitis.

Keywords: pregnancy, periodontitis, diabetes, saliva, MMP-9

Introduction

Periodontitis is a common chronic bacterial inflammatory disease.[1] It is an infectious disease that affects the soft tissues surrounding the teeth, and leads to progressive bone loss and clinical attachment loss (CAL). As periodontitis progresses, periodontal pockets develop, which may further advance to tooth displacement, tooth loosening, and eventually tooth loss. Periodontitis is commonly accompanied by gingival bleeding and halitosis.[2]

Current knowledge indicates that periodontitis is a multifactorial disease that results from the interactions between individual host-related factors and environmental factors.[3] Periodontitis is initiated as a primary inflammatory reaction to pathogens, and bacterial lipopolysaccharides, collagenases, capsular polysaccharides, and fimbriae; it is sustained by their constant presence as dental plaque biofilm.[4] As a result, CAL occurs in periodontal tissues, causing the development of periodontal pockets that are further colonized by bacteria. The secondary inflammatory response of the gingiva involves the gathering of immune cells, and the production of interleukins (ILs), proteases, tumor necrosis factor alpha (TNF-α), and C-reactive protein (CRP).[5] Proteases constitute a large group of enzymes that include matrix metalloproteinases (MMPs). The essential contribution of MMPs in periodontal remodeling involves the vital pathways of tissue destruction, collagen proteoglycans and the degradation of other matrix components.[6,7] Metalloproteinase-9 (MMP-9), which is a product of neutrophilic white blood cells, plays a crucial role in connective tissue decay in periodontal diseases. High MMP-9 concentration in periodontal tissues leads to CAL, weaker tissue structure and delayed healing, which are more prominent in people with chronic forms of periodontitis than in healthy adults.[8]

Furthermore, the inflammatory process triggers the production of reactive oxygen species (ROS) by host cells in response to bacterial challenges, which constitutes an important defense mechanism.[9] Increased ROS levels may also cause tissue and structural damage, and contribute to the development of CAL.[10] Along with the inflammatory process, protective mechanisms are activated, including the production of salivary antioxidants – catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), and glutathione peroxidase (GPX) –to reduce the potential tissue damage.[11,12]

The major antioxidants include SOD, which inactivates superoxide anion radicals in the extracellular space,[13] glutathione (GSH), which is considered one of the most important antioxidants involved in the inflammatory process, GPX, which takes part in the reduction of hydrogen peroxide with the simultaneous conversion of reduced glutathione into its oxidized form, and GR, which maintains normal GSH concentration in the cell due to its ability to convert oxidized glutathione (GSSG) into its reduced form.[14] It is possible to determine the activity of individual antioxidants as well as the overall antioxidant capacity. Determining the total antioxidant capacity (TAC) allows researchers to assess the activity of the aforementioned antioxidants in addition to numerous other molecules that have antioxidant properties. The method is used to test the antioxidant capacity of biological samples and to demonstrate the ability of the organism to destroy ROS. The interaction between different antioxidants often results in better protection than might be expected from the antioxidant properties of individual compounds.[11]

The clinical effect of periodontitis results from a balance between the severity of bacterial colonization, the inflammatory process and defensive mechanisms.[3]

It is essential for pregnant women to receive dental, particularly periodontal, health care.[15] Pregnancy is associated with the risk of progression of prior periodontitis or the development of pregnancy gingivitis, which is most severe during the 3rd trimester of pregnancy. These diseases are associated with numerous complications, including preeclampsia, preterm birth and low birth weight.[16] Periodontitis is further aggravated by diabetes, which decreases the neutrophil activity in the area of the periodontal pocket and impedes the wound healing process due to collagen homeostasis disorders, the formation of advanced glycation end-products (AGE) and the release of excess proinflammatory cytokines.[17]

The diagnosis of periodontitis in pregnant women is challenging due to limitations in X-ray exposure, and consequently radiological imaging. Despite the fact that the amount of radiation in a dental X-ray is very low and insufficient to have adverse effects on a developing fetus, pregnant women often have concerns about taking X-rays.[18] Therefore, the potential investigations to obviate the need for radiological exposure in order to diagnose periodontitis are of significant clinical value. Salivary sample collection is easy and not time-consuming; if properly assessed, saliva samples can be useful to support standard periodontal examinations in diagnosing periodontitis.

Therefore, the aim of the present study was to assess the health status of the gingiva and periodontium of pregnant women with regard to the presence of 2 different forms of diabetes. Additionally, we aimed to assess antioxidant activity and the level of MMP-9 in saliva in order to establish the optimal noninvasive determinants of periodontitis.

Material and methods

Patients

The study participants were recruited between March 2014 and November 2015 from among pregnant women diagnosed with diabetes and treated in the Department of Metabolic Diseases of the Jagiellonian University Medical College, Cracow, Poland. In terms of numbers, the matched control group participants were recruited during the same period amongst pregnant women without diabetes from the Department of Gynecology and Obstetrics of Jagiellonian University Medical College. The patients were eligible to participate in the study if they: (1) provided informed consent; (2) were over 18 years of age; and (3) were in the 3rd trimester of pregnancy. The diagnoses of gestational diabetes mellitus (GDM) or type 1 diabetes (T1D) were made by the principal physician according to the guidelines of the Polish Diabetes Association (Diabetes Poland).[19] The patients were excluded if they: (1) did not provide informed consent; (2) had other tpes of diabetes; (3) had salivary gland diseases that could potentially cause salivary excretion disturbances; (4) were diagnosed with other metabolic conditions affecting the metabolism of carbohydrates; (5) had a primary or secondary immunodeficiency disorder; (6) presented with an active inflammatory or infectious process; or (7) were taking medications that could affect the biochemical composition of their saliva. The study protocol was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional Ethics Committee at Jagiellonian University Collegium Medicum (KBET/270/B/2013). Informed consent was obtained from each patient prior to participation in the study.

A medical history was obtained from each patient based on medical records. Each patient underwent an oral examination and saliva samples were collected at the same time. Data collection included the information regarding concomitant diseases, medications, the course of diabetes, the current glikemia, a family history of diabetes, and the presence and type of oral symptoms.

Clinical assessment

An experienced dentist (J.W.) carried out full-mouth examinations in each subject. The physical examination was performed using a dental mouth mirror and a fixed periodontal probe (PCP12; Hu-Friedy, Chicago, USA). The oral examination included the assessment of the gums and periodontium, and the measurements of the following parameters: gingival index (GI) according to Löe and Silness; bleeding on probing (BOP); probing pocket depth (PPD); and CAL.

The GI assessment was based on the visual features of gingivitis, and the presence of bleeding from 6 selected teeth (16, 12, 24, 36, 32, and 44) on the vestibular, lingual, mesial, and distal surfaces. In the case of the absence of any of the aforementioned teeth, the condition of the gums at the next adjacent tooth was determined. The condition of the gums was graded from 0 to 3 for each side, and then the average value of the index for each tooth was calculated. The mean value for each patient was calculated from the sum of the dental index scores divided by their number. The higher the value, the more severe gingivitis.[20] The BOP was determined with the use of a periodontal probe; it was defined as the presence of any bleeding during the probing of the periodontal pocket at 6 points (the buccal-mesial, midbuccal, buccal-distal, lingual-mesial, midlingual, and lingual-distal sites) for each tooth and presented as a percentage. The presence of bleeding was recorded for up to 30 s after probing.[21]

The PPD was evaluated for each tooth with a periodontal probe by measuring the distance between the gingival margin and the bottom of the periodontal pocket. The measurement was performed parallel to the long axis of each tooth, in direct contact with the root of the tooth. The CAL was measured for each tooth with a periodontal probe as a distance between the bottom of the pocket and the cementoenamel junction. The PPD and CAL measurements were made at 6 points (the buccal-mesial, midbuccal, buccal-distal, lingual-mesial, midlingual, and lingual-distal sites), and the mean values were calculated for each tooth, except for third molars. The averages of the PPD and CAL values for each patient were calculated and expressed in millimeters.

The approximal plaque index (API) was defined as the number of interdental spaces with the presence of dental plaque divided by the number of all spaces assessed; it was expressed as a percentage. In quadrants I and III, the examination was conducted on the lingual side, whereas in quadrants II and IV, the examination was conducted on the buccal side.[20]

Periodontal health was assessed based on the report of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions.[22] Periodontally healthy women had BOP < 10% and no sites with PPD > 3 mm. Gingivitis was diagnosed as having BOP ≥ 10% and PPD ≤ 3mm. The higher the BOP, the more severe the inflammation.[22] Periodontitis was diagnosed if: (1) interdental CAL was detectable at 2 or more non-adjacent teeth; or (2) buccal/oral CAL ≥ 3 mm with PPD > 3 mm was detectable at 2 or more teeth, and the observed CAL could not be ascribed to non-periodontal causes.[23] Additionally, tooth loss due to periodontitis was assessed, while alveolar bone loss on radiography was not assessed.

The fasting plasma glucose (FPG) levels were determined on the day of the dental examination.

Saliva collection

Two samples of 1 mL of unstimulated saliva were collected from each patient. The sampling was performed between 8 a.m. and 11 a.m., at least 1 h after the last meal and before the dental examination so that to avoid blood contamination. The calibrated tubes were filled with saliva freely flowing from the mouth. The samples were immediately centrifuged at 10,000 g for 5 min at 4°C, and then frozen and stored at −80°C until the sample collection period was completed; they were thawed immediately before the assays were performed.

Saliva analysis

The analysis included the assessment of MMP-9 concentration, total antioxidant capacity (TAC), and the extracellular SOD activity, the GR activity and the GPX activity.

The concentration of MMP-9 was measured using the enzyme-linked immunosorbent assay (ELISA) (Quantikine® Human MMP-9 ELISA Kit; R&D Systems, Inc., Minneapolis, USA) and a micro-ELISA reader (ELx808™ Absorbance Microplate Reader; BioTek Instruments, Winooski, USA) according to the instructions provided by the manufacturers. The samples were analyzed in duplicate, immediately after the collection of all samples. The saliva samples were thawed and diluted at least 100-fold with Calibrator Diluent RD5-10, as recommended by the manufacturer. Due to the high concentration of MMP-9 found in saliva, the measurements were performed using face masks and gloves, with special attention paid to preventing the contamination of the samples and the reagents with the researcher's saliva. According to the manufacturer, the minimum detectable dose of human MMP-9 is typically less than 0.156 ng/mL.

The SOD activity was measured using the Misra and Fridovich method, which is based on the inhibition of the auto-oxidation of adrenaline to adrenochrome at alkaline pH.[24] The measurement was conducted at wavelength $\lambda = 480$ nm.

Total antioxidant capacity was measured using the Benzie and Strain ferric reducing ability of plasma (FRAP) method.[25] It was based on the assessment of the ability to reduce Fe^{3+} ions present in a complex form with tripyridyltriazine (Fe^{3+}-TPTZ) by the low-molecular-weight antioxidants contained in the test biological material. The resulting Fe^{2+}-TPTZ complex is characterized by an intense blue color and has a maximum absorption at wavelength $\lambda = 593$ nm.

The modified Goldberg method[26] was used to measure the GR activity. Glutathione reductase catalyzes the reduction of GSSG in the presence of nicotinamide adenine dinucleotide phosphate (NADPH), which is oxidized to $NADP^+$. A decrease in the NADPH absorbance was measured at 412 nm.

The GPX activity was measured using the method described by Paglia and Valentine.[27] Glutathione peroxidase is a catalyst for the oxidation of GSH. In the presence of GR and NADPH, GSSG is immediately converted to its reduced form with the simultaneous oxidation of NADPH to $NADP^+$. A decrease in the NADPH absorbance was measured at 340 nm.

The antioxidant analysis was performed using the ELx808 Absorbance Microplate Reader (BioTek Instruments).

The measurements were performed at the Department of Diagnostics, Chair of Clinical Biochemistry, Jagiellonian University Medical College, Cracow, Poland.

Statistical analysis

Statistical analysis was performed using the STATISTICA PL data analysis software, v. 9.1 (StatSoft Polska, Cracow, Poland), and the MedCalc® program, v. 8.1.1.0 (https://www.medcalc.org/). Continuous variables are shown as median and interquartile range (*Me* (*IQR*)), and categorical variables as number and percentage (*n* (%)).

The parameters were compared between the patients with GDM, T1D and the control group. The Kruskal–Wallis test was used to compare continuous variables. The χ^2 test was used for dichotomous variables. One repetition was performed for each measurement.

A logistic regression model with stepwise selection was used to assess the factors determining the presence of periodontitis. Variables for which the *p*-value was <0.20 were included. The results are presented with odds ratios (*OR*s) and confidence intervals (*CI*s). The level of significance was set at $p < 0.05$.

The receiver operating characteristics (ROS) analysis was performed to calculate the area under the curve (AUC), which describes the accuracy of a test, to discriminate periodontitis from healthy cases. The clinical signs of periodontitis were the reference standard against which the AUC of the MMP-9 level was assessed. For criteria with significant discriminative capacity, we calculated the sensitivity and specificity of the currently recommended cut-off values. Additionally, the cut-off values with the highest sensitivity and specificity were established for these criteria. The significance level was established at $p < 0.05$.

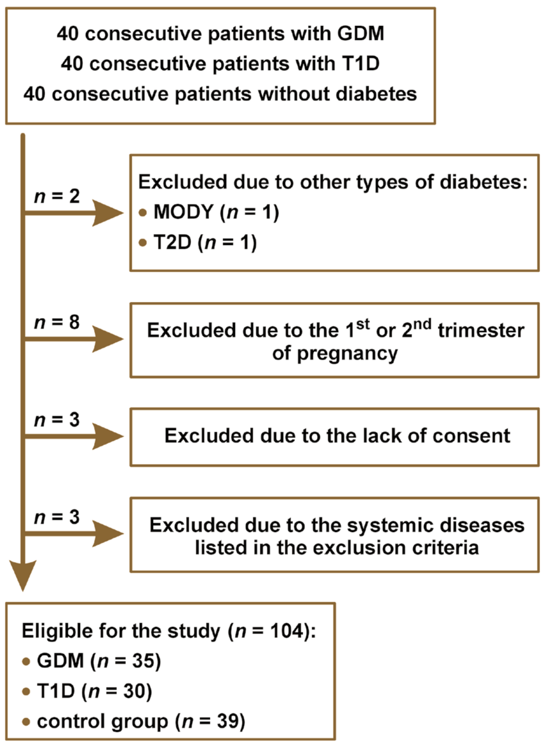

Fig. 1. Flowchart representing the inclusion and exclusion criteria for the study

GDM – gestational diabetes mellitus; T1D – type 1 diabetes; MODY – maturity-onset diabetes of the young; T2D – type 2 diabetes.

Results

Study group

Out of 40 consecutive patients for each study group, 104 met the inclusion criteria: 35 patients with GDM; 30 patients with T1D; and 39 patients without diabetes (the control group), as presented in the flowchart (Fig. 1). The groups did not differ in terms of age, height, body weight before pregnancy, body mass index (BMI) before pregnancy, body weight during pregnancy, number of pregnancies, number of miscarriages, prevalence of GDM in previous pregnancies, and prevalence of diabetes in the family. Hypothyroidism and Hashimoto thyroiditis were present more frequently in the patients with T1D than in the patients with GDM and in the control group (11 (36.7%) vs. 4 (11.4%), $p = 0.0200$; 11 (36.7%) vs. 6 (15.4%), $p = 0.0001$; and 10 (33.3%) vs. 1 (2.9%), $p = 0.0010$; 10 (33.3%) vs. 0 (0%), $p = 0.0400$, respectively). There were no differences between the groups in the frequency of other diseases. The concentration of FPG [mmol/L] was the highest in the patients with T1D, lower in the patients with GDM and the lowest in the control group (6.22 (5.16–7.55) vs. 4.77 (4.50–5.16) vs. 4.34 (4.14–4.82), respectively; $p < 0.0001$). The glycated hemoglobin (HbA1c) level in the T1D patients was 5.75% (5.10–6.10%). The baseline characteristics of the study groups are presented in Table 1.

Table 1. Baseline characteristics of the study groups

Variable		All participants *N* = 104	Control group *n* = 39	GDM group *n* = 35	T1D group *n* = 30	*p*-value
Anthropometric data	age [years]	31.0 (28.0–33.0)	30.0 (26.0–33.0)	32.0 (29.0–34.0)	31.0 (28.0–33.0)	0.3900
	height [cm]	165.0 (161.0–170.0)	165.0 (162.0–169.0)	164.0 (160.0–168.0)	167.5 (163.0–170.0)	0.1200
	body weight before pregnancy [kg]	63.0 (56.5–69.0)	62.0 (57.0–65.0)	65.0 (56.0–74.0)	61.5 (54.5–73.0)	0.4900
	BMI before pregnancy [kg/m^2]	22.7 (21.6–25.2)	22.8 (21.8–23.6)	23.8 (21.6–27.5)	22.2 (20.8–25.9)	0.1700
	body weight during pregnancy [kg]	73.6 (68.0–82.2)	73.6 (69.6–80.5)	74.5 (65.5–85.5)	73.5 (68.0–85.8)	0.9000
Medical history	twin pregnancy *n* (%)	5 (4.8)	3 (7.7)	1 (2.9)	1 (3.3)	0.3300
	first pregnancy *n* (%)	42 (40.4)	16 (41.0)	14 (40.0)	12 (40.0)	0.9900
	prior miscarriage *n* (%)	21 (20.2)	9 (23.1)	7 (20.0)	5 (16.7)	0.8100
	prior GDM *n* (%)	8 (7.7)	2 (5.1)	5 (14.3)	1 (3.3)	0.1900
	prevalence of diabetes in the family *n* (%)	50 (48.1)	16 (41.0)	18 (51.4)	16 (53.3)	0.5300
	hypothyroidism *n* (%)	21 (20.2)	6 (15.4)	4 (11.4)	11 (36.7)†‡	0.0300*
	Hashimoto disease *n* (%)	11 (10.6)	0 (0)	1 (2.9)	10 (33.3)†‡	0.0001*
	hypothyroidism diagnosed during pregnancy *n* (%)	6 (5.8)	0 (0)	2 (5.7)	4 (13.3)	0.0600
	hypertension *n* (%)	3 (2.9)	1 (2.6)	2 (5.7)	0 (0.0)	0.3900
	hypertension diagnosed during pregnancy *n* (%)	7 (6.7)	3 (7.7)	3 (8.6)	1 (3.3)	0.6700
	intrahepatic cholestasis of pregnancy *n* (%)	3 (2.9)	3 (7.7)	0 (0)	0 (0)	0.0900
	migraine *n* (%)	1 (1.0)	0 (0)	1 (2.9)	0 (0)	0.3700
Laboratory tests	FPG level [mmol/L]	4.82 (4.32–5.39)	4.34 (4.14–4.82)	4.77 (4.50–5.16)†	6.22 (5.16–7.55)†‡	<0.0001*

Data presented as median (interquartile range) (*Me* (*IQR*)), or as number (percentage) (*n* (%)). BMI – body mass index; FPG – fasting plasma glucose; * statistically significant; † statistically significant difference as compared to the control group ($p < 0.05$); ‡ statistically significant difference between the GDM and T1D groups ($p < 0.05$).

Health status of the gingiva and periodontium

The patients with GDM and T1D and those in the control group did not differ in terms of frequency of occurrence and type of self-reported oral conditions, such as gum bleeding during brushing, tooth and/or gum hypersensitivity, or halitosis. The most frequently reported symptom was gum bleeding during brushing, which was present in almost half of the subjects ($n = 50$; 48.1%), while other symptoms, including tooth and/or gum hypersensitivity ($n = 14$; 13.5%) and halitosis ($n = 2$; 1.9%), were infrequent. None of the patients had a history of periodontitis or periodontal treatment. No tooth loss due to periodontitis was observed. The data regarding the self-reported oral conditions is shown in Table 2.

The pregnant women with GDM had significantly higher markers of gingivitis and periodontitis, as measured by the GI, BOP, PPD, and CAL scores ($p < 0.0001$, $p = 0.0040$, $p = 0.0100$, and $p = 0.0030$, respectively), but not oral hygiene, as measured by API ($p = 0.0900$), as compared to the control group participants. The T1D patients had significantly higher markers of gingivitis, periodontitis and oral hygiene, as measured by the GI, BOP, PPD, CAL, and API scores, as compared to the control group subjects ($p < 0.0001$, $p < 0.0009$, $p < 0.0001$, $p < 0.0001$, and $p = 0.0010$, respectively). In addition, the markers of periodontitis – PPD and CAL – were significantly higher in the T1D group as compared to the GDM group ($p = 0.0060$ and $p = 0.0010$, respectively).

Gingivitis was present in the majority of the participants ($n = 75$; 72.12%) and its prevalence did not differ among the study groups (27 (77.15%) in the GDM group, 19 (63.33%) in the T1D group and 29 (74.36%) in the control group; $p = 0.4300$). However, differences between the groups regarding the GI and BOP scores indicated that gingivitis was more severe in the pregnant women with GDM and T1D than in the controls.

The prevalence of periodontitis in the entire sample was 21.15% ($n = 22$); no difference was observed between the groups ($p = 0.0600$). However, an upward trend in periodontitis frequency in the control group, the GDM group and the T1D group is worth noting (10.26% vs. 22.86% vs. 33.33%, respectively). The minority of patients had an acceptable gum and periodontium condition ($n = 7$; 6.73%). However, it should be noted that an acceptable gum and periodontium condition was found most often in the control group ($n = 6$; 15.38%), while in the GDM ($n = 0$, 0%) and T1D ($n = 1$; 3.33%) groups, a satisfactory gum and periodontium condition was rare ($p = 0.0200$). Clinical periodontal markers, oral hygiene markers and the periodontal health diagnosis are outlined in Table 3.

Table 2. Dental interview data – self-reported oral conditions

Variable	All participants $N = 104$	Control group $n = 39$	GDM group $n = 35$	T1D group $n = 30$	p-value
Gum bleeding during brushing	50 (48.1)	15 (38.5)	19 (54.3)	16 (53.3)	0.3100
Teeth and/or gums hypersensitivity	14 (13.5)	6 (15.4)	3 (8.6)	5 (16.7)	0.5800
Halitosis	2 (1.9)	1 (2.6)	0 (0)	1 (3.3)	0.1200

Data presented as *n* (%).

Table 3. Clinical markers of the oral health status and hygiene, and the periodontal health diagnosis

Indicators and diagnosis	All participants $N = 104$	Control group $n = 39$	GDM group $n = 35$	T1D group $n = 30$	p-value
GI	1.05 (0.77–1.22)	0.80 (0.53–0.97)	1.10 (0.90–1.25)†	1.22 (0.86–1.86)†	<0.0001*
BOP [%]	45 (32–65)	36 (28–48)	50 (35–62)†	55 (37–90)†	0.0200*
PPD [mm]	2.1 (2.0–2.3)	2.0 (1.8–2.1)	2.1 (2.0–2.3)†	2.3 (2.1–2.8)†‡	0.0002*
CAL [mm]	2.2 (2.0–2.5)	2.1 (1.8–2.3)	2.2 (2.0–2.4)†	2.5 (2.2–2.9)†‡	0.0100*
API [%]	50 (38–79)	46 (31–64)	50 (39–66)	69 (44–97)†	0.0300*
Gingivitis *n* (%)	75 (72.12)	29 (74.36)	27 (77.15)	19 (63.33)	0.4300
Periodontitis *n* (%)	22 (21.15)	4 (10.26)	8 (22.86)	10 (33.33)	0.0600
Periodontal and gingival health *n* (%)	7 (6.73)	6 (15.38)	0 (0)†	1 (3.33)†	0.0200*

Data presented as *Me* (*IQR*) or as *n* (%). GI – gingival index; BOP – bleeding on probing; PPD – probing pocket depth; CAL – clinical attachment loss; * statistically significant; † statistically significant difference as compared to the control group ($p < 0.05$); ‡ statistically significant difference between the GDM and T1D groups ($p < 0.05$).

Antioxidant activity and the level of MMP-9 in saliva

Salivary MMP-9 concentration was higher in the pregnant women with T1D than in the controls, but it did not differ between the patients with GDM and the controls. The patients with periodontitis had higher concentration of MMP-9 [μg/mL] in their saliva than the patients without periodontitis (2.29 (1.10–3.04) vs. 0.88 (0.50–1.84); $p = 0.0001$). Salivary antioxidant concentration/activity was comparable among the study groups. The levels of the examined salivary biomarkers are shown in Table 4.

The independent determinants of periodontitis ($p < 0.0001$ for the logistic regression model; $R^2 = 0.46$) were MMP-9 concentration (*OR* = 2.92; 95% *CI*: 1.56–5.46; $p = 0.0008$) and oral hygiene based on API (*OR* = 1.05; 95% *CI*: 1.03–1.08; $p = 0.0001$). In addition, MMP-9 concentration was also a useful determiner of the presence of periodontitis (ROC AUC = 0.77; 95% *CI*: 0.68–0.85; $p < 0.0001$). The optimal cut-off point was 0.84 μg/mL, with a sensitivity of 95.5% and a specificity of 47.6%. A graphical delineation of the ROC curve is presented in Fig. 2.

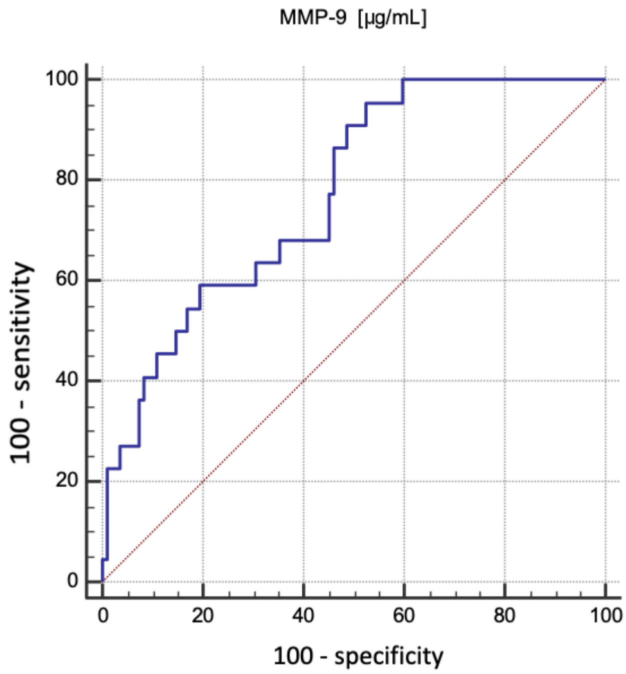

Fig. 2. Receiver operating characteristic (ROC) curve of metalloproteinase-9 (MMP-9) concentration as a periodontitis predictor (AUC: 0.77; 95% *CI*: 0.68–0.85; $p < 0.0001$)

Discussion

In this study, we demonstrated that the prevalence of gingivitis in pregnant women was high, affecting as much as 72.12% of the examined patients. A proper condition of the gingiva was observed most often in the controls. Additionally, we showed that the antioxidant levels in saliva were comparable in the study groups, but the concentration of MMP-9 in the T1D group was higher as compared to the controls. In all 3 study groups, the patients with periodontitis had higher concentration of MMP-9 in their saliva as compared to the patients without periodontitis. The independent determinants of periodontal disease in our study were oral hygiene and MMP-9 concentration.

Gingivitis

The prevalence of gingivitis in the study groups was 72.12%; gum bleeding during brushing, which is one of the symptoms of gingivitis, was the most frequently self-reported oral condition (48.1%).

Gingivitis during pregnancy is associated with some hormonal changes that occur in the woman's body. Changes in the serum concentration of estrogen and progesterone can lead to gingival edema and gingivitis in 50% of pregnant women.[28] The presence of female sex hormone receptors in the gingiva may explain an enhanced gingival response to plaque, and greater susceptibility to gingival and periodontal diseases during pregnancy.[28] According to most studies, the severity of gingivitis increases with the duration of pregnancy,

Table 4. Comparison of the salivary levels of antioxidants and metalloproteinase-9 (MMP-9)

Variable	All participants	Control group	GDM group	T1D group	*p*-value
FRAP [mmol/L]	0.42 (0.32–0.53)	0.41 (0.33–0.55)	0.42 (0.30–0.51)	0.43 (0.28–0.56)	0.9700
SOD [U/mL]	12.8 (10.7–15.7)	14.1 (10.9–17.7)	12.5 (9.5–14.2)	12.6 (10.6–15.3)	0.3800
GR [U/L]	9.9 (5.4–14.2)	11.5 (5.7–16.4)	10.3 (6.2–15.8)	7.9 (4.7–10.7)	0.2200
GPX [U/mL]	29.3 (14.5–71.4)	37.2 (15.1–67.0)	25.8 (11.7–71.0)	27.1 (19.1–96.3)	0.7800
MMP-9 [μg/mL]	1.12 (0.58–2.15)	0.85 (0.42–1.40)	1.10 (0.50–2.25)	1.60 (1.08–2.62)†	0.0200*

Data presented as *Me* (*IQR*). FRAP – total antioxidant capacity (TAC), as measured with the ferric reducing ability of plasma method; SOD – extracellular superoxide dismutase; GR – glutathione reductase; GPX – glutathione peroxidase; * statistically significant; † statistically significant difference as compared to the control group ($p < 0.05$).

and then decreases during the postpartum period. This may explain a high prevalence of gingivitis in our study group, which is comparable to that reported by Hassan et al.[29] and Weintraub et al.,[30] i.e., 72% and 69%, respectively.

The groups did not differ in terms of gingivitis prevalence, but higher GI and BOP scores indicate that moderate to severe inflammation prevailed in the groups with diabetes, while mild gingivitis was observed in the control group, which is consistent with other studies; Ruiz et al. observed that the GI, BOP, PPD, and CAL scores were significantly higher in GDM and T1D groups as compared to controls,[31] and Mittas et al. reported that GDM patients in the 3rd trimester had more severe gingivitis than pregnant women without diabetes.[32]

Periodontitis and diabetes

Despite differences in the values of indicators such as GI, BOP, PPD, and CAL, no differences were observed between the groups in the prevalence of periodontitis.

There is no definitive evidence that GDM causes periodontitis, as GDM is only the early stage of abnormal glucose tolerance. Gestational diabetes mellitus frequently occurs late in pregnancy, is transient, and then normalizes after childbirth. Therefore, the period of hyperglycemia is short, and often so mild that it may not have a significant effect on tissue loss in the periodontium.[32] However, most reports from the literature indicate that there is a relationship between gingivitis and the exacerbation of periodontal disease that was present before pregnancy in patients who eventually develop GDM.[31,32] In our study, the presence of diabetes was not a predictor of periodontitis.

Despite a recent increase in the number of studies associating periodontitis with diabetes, there is a paucity of reports regarding the relationship between periodontal disease and T1D.[33] However, most authors report that there is an association between these two states, and changes in the periodontium refer mainly to patients with poorly controlled glycemia. Thus, the degree of changes in the periodontium is related to the duration of diabetes. For this reason, the prevalence of periodontal disease increases in older adults with diabetes.[17] All of the patients in our study were young and of a similar age (*Me*: 31 years). We did not find a clear association between diabetes and periodontitis. The logistic regression model omitted diabetes, possibly due to the proper control of diabetes in the study groups. In T1D, normoglycemia was monitored using the concentration of HbA1c, as recommended by standards,[19] and most participants properly controlled their diabetes (5.75% (5.10–6.10%)). In GDM, there were no routine HbA1 measurements performed, as there are no standard recommendations for such monitoring.

Periodontitis and other factors

In our study, the patients' concomitant diseases, such as hypothyroidism, Hashimoto disease, hypertension, intrahepatic cholestasis of pregnancy, and migraine, were not associated with the presence of periodontal disease. Moreover, the reported effect of these diseases on dental health is controversial in the available studies, and little is known about their possible cause-and-effect relationship with periodontitis.

In a systematic review by Aldulaijan et al., the authors hypothesized about an uncertain link between periodontitis and hypothyroidism, and they speculated that hypothyroidism might be more apparent in individuals with more severe periodontitis.[34] However, this opinion is based only on several studies with multiple limitations.[34]

Recent studies revealed a relationship between periodontitis and migraine headaches. Inflammatory processes as well as vascular endothelial changes could be the potential mediators of this association. Leira et al. observed higher serum procalcitonin levels in patients with periodontitis and chronic migraines as compared to controls.[35]

The systemic inflammatory response in periodontitis may have adverse effects on blood pressure. Periodontal pathogens can cause transient bacteremia, which may lead to vascular inflammation, endothelial dysfunction, and ultimately hypertension.[36]

Currently, there is no data regarding a possible cause-and-effect relationship between intrahepatic cholestasis of pregnancy and periodontal disease.

Antioxidants in saliva

The antioxidant levels in saliva were comparable between the groups. An increase in oxidative stress is followed by a rise in the total level of salivary antioxidants to balance this stress. In diabetes, oxidative stress is observed mainly due to poor glycemic control, so it is likely that the patients in the GDM and T1D groups had their diabetes under proper control. However, in a study by Zamani-Ahari et al., the TAC level in the saliva of women with GDM was higher than in pregnant women without diabetes.[37] Two possible explanations for this discrepancy are differences in the laboratory testing methods used and in pregnancy trimesters.

In a study by Zygula et al., patients with GDM who received only nutritional therapy had lower oxidative stress levels than patients with GDM who received insulin treatment.[38] Patients with diabetes, especially those on nutritional therapy, often have a balanced, low-calorie diet, which may cause changes in the level of oxidative stress and antioxidant activity.

Metalloproteinases in saliva

The salivary concentration of MMP-9 in the T1D group was higher than in the controls ($p = 0.0010$), but its concentration did not differ between the patients with GDM and the controls. Similarly, Caseiro et al. found that the salivary MMP-9 levels were higher in patients who had T1D and periodontitis as compared to controls.[39] Both studies suggest that high salivary MMP-9 concentration in T1D patients is related to deeper periodontal pockets and greater CAL. Considering that there is limited scientific data regarding the relationship between salivary MMP-9 concentration and pregnancy, GDM and T1D, our study provides some important insights into these populations. The only available data concerning MMP-9 concentration in pregnancy comes from a study by Öztürk et al., who observed that the MMP-9 level in saliva was higher in pregnant women than in non-pregnant women, and that it could be used to monitor the inflammatory state of gingival tissues during pregnancy.[40] In Akcalı et al.'s study, elevated MMP-9 concentration was observed in the gingival pocket fluid of patients with GDM and gingivitis as compared to women with GDM who had healthy gums and periodontium.[41]

Antioxidants and periodontitis

Antioxidant activity was at a similar level in all study groups; we found no association between antioxidant activity and periodontitis. Although some literature reports indicate that oxidative stress contributes to periodontal disease,[42] the vast majority of data on antioxidant activity in saliva is contradictory. However, it is possible that antioxidants might affect many processes that are not directly related to the action of free radicals. Throughout the course of periodontitis, both increases and decreases in the activity and concentration of several antioxidants can been observed, especially in gingival fluid and saliva.[11] The evaluation of TAC seems particularly useful due to the interaction and synergistic effects between antioxidants. According to most studies on patients with periodontitis, their antioxidant status was lower as compared to controls without periodontitis; it is likely that the TAC of saliva was reduced because of chronic inflammation in periodontal tissues.[12,43] However, some studies reported that the SOD, GR and GPX activity was higher in the saliva of patients with periodontitis as compared to that of controls.[44,45] Differences in the results of the aforementioned studies could be related to differences in methodologies, including sampling, laboratory testing, and groups that were included. One possible explanation is that there are other currently unestablished factors that contribute to the development of periodontitis.

MMP-9 and periodontitis

In this study, the independent determinants of periodontal disease included oral hygiene and MMP-9 concentration. Each increase in MMP-9 concentration by 1 μg/mL increased the prevalence of periodontitis by 192%, regardless of the oral hygiene records (an increase in API by 1% increases this chance by 5% independently). This model explains almost half of the variability in the presence of periodontitis.

Many studies proved a direct correlation between the presence of periodontitis and the amount of dental plaque. The bacteria contained in dental plaque initiate and support periodontitis.[2,4] Further advancement of the inflammatory cascade is affected by the factors derived from the host; one such factor could be MMP-9.[46] This enzyme is capable of extracellular matrix protein degradation and is a mediator of tissue breakdown in periodontitis. Elevated levels of MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 were detected in the gingival pocket fluid, gingival tissue and saliva of patients with periodontitis.[47,48] In a study by Wu et al., biomarkers such as IL-1β, MMP-8 and MMP-9 showed the potential to identify patients with periodontitis.[8] The levels of IL-1β and MMP-9 were significantly higher in the periodontitis group. In a prediction model for diagnosing periodontitis, a combination of 3 biomarkers (IL-1β, IL-1ra and MMP-9) exhibited the highest AUC (0.853), with high sensitivity (73.3%) and specificity (88.9%).[8]

Some studies showed that combining bacterial and host-derived salivary biomarkers, such as MMP-8 and MMP-9, could be considered a potential diagnostic tool for predicting periodontal disease. According to Ramseier et al., the combination of MMP-8, MMP-9 and red-complex anaerobic periodontal pathogens was a good prediction model for diagnosing periodontitis.[49] In Salminen et al.'s study, the levels of IL-1β, MMP-8 and *Porphyromonas gingivalis* in saliva were associated with periodontitis.[4] Our study supports this assumption by providing evidence for the use of MMP-9 concentration as a marker of periodontitis.

Finally, we showed that the MMP-9 level is an independent and useful marker of gingivitis. We determined that gingivitis was more intense in the patients with GDM than in the control group, despite a similar level of hygiene, as represented by API. This may be due to the fact that other factors play major roles in the development of gingivitis. One possible underlying cause involves the host-related factors associated with inflammatory progression. Based on Kinney et al.'s study,[50] we hypothesized that these factors include proteases, such as MMP-9.

Limitations

This study has limitations. The main limitation is the lack of the assessment of the presence and advancement of periodontal disease on dental X-rays. This is limited in pregnant women by 3 major factors. First, patients are unwilling to undergo X-ray examinations. Second, X-ray imaging is subjected to medical restrictions, except for the most urgent cases; otherwise, they are frequently delayed until after childbirth. Third, performing an X-ray examination only for the purpose of this study would be ethically questionable.[29] Another limitation is that only a few salivary biomarkers were included in this study, so further research is warranted.

Conclusions

We observed worse gingival conditions, deeper periodontal pockets and greater attachment loss in the pregnant women with diabetes in comparison with the pregnant women without diabetes. However, we found no association between antioxidant activity and periodontitis in the gestational subgroups. We established that oral hygiene and MMP-9 concentration were the determinants of periodontal disease. Then, we established that MMP-9 concentration was a good predictor of periodontitis, as determined using the ROS analysis. Finally, we established a novel cut-off point for MMP-9 of 0.84 µg/mL to diagnose periodontitis.

Ethics approval and consent to participate

The study protocol was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional Ethics Committee at Jagiellonian University Collegium Medicum, Cracow, Poland (KBET/270/B/2013). Informed consent was obtained from each patient prior to participation in 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.

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Original paper

Association of breastfeeding duration with the development of non-nutritive habits, and transversal and vertical occlusal alterations in preschool children: A cross-sectional study

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Abstract

Background. Breastfeeding has multiple nutritional, immunological and psychological benefits, as well as a positive influence on the development of the stomatognathic system.

Objectives. The present study attempted to determine the relationship between the duration of breastfeeding and the development of non-nutritive habits, and transversal and vertical occlusal alterations in preschool children.

Material and methods. This cross-sectional study involved 155 preschoolers aged 2–5 years from 3 public schools in Lima, Peru. The sample was divided into 2 groups with regard to the duration of breastfeeding: group A ($n = 50$) included infants that had been breastfed for up to 6 months; and group B ($n = 105$) included infants that had been breastfed for 6–12 months. Two trained and calibrated dentists evaluated each group, and clinically determined the presence or absence of transversal or vertical occlusal alterations. Likewise, the children's parents or caregivers were consulted to determine the development of non-nutritive habits.

Results. A significant association was found between breastfeeding duration and the development of the oral breathing habit; groups A and B showed a prevalence of 30.0% and 16.2%, respectively ($p = 0.048$). Likewise, breastfeeding duration affected the appearance of oral breathing (*OR* (odds ratio) = 0.84; 95% *CI* (confidence interval): 0.74–0.96; $p = 0.011$). Furthermore, the use of a bottle for more than 24 months significantly influenced the appearance of oral habits (*OR* = 3.55; 95% *CI*: 1.20–10.55; $p = 0.022$) and open bite (*OR* = 12.12; 95% *CI*: 1.16–126.31; $p = 0.037$).

Conclusions. Breastfeeding duration of 6–12 months was shown to be a protective factor in preventing the appearance of oral breathing. Posterior crossbite (PCB), open bite and deep bite seemed not to be influenced by breastfeeding duration. However, the use of a bottle for more than 24 months significantly influenced the appearance of anterior open bite.

Keywords: habits, breastfeeding, dental occlusion

Introduction

Breastfeeding is the first step in promoting the general health of both babies and their mothers.[1] Breastfeeding has multiple nutritional, immunological and psychological benefits, as well as a positive influence on the development of the stomatognathic system.[2–11] The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) recommend an early start for breastfeeding (within the first hour of life), exclusive breastfeeding for the first 6 months of life, and continued breastfeeding for up to 24 months or more in order to achieve optimal growth, development and health.[12–18]

Breast milk is best for babies because of its immunomodulatory effects and protection against early infections. Early infections are major risk factors for asthma and allergic diseases; protection through breastfeeding may be a pathway that shields against allergic diseases.[19]

Breastfeeding reinforces the physiological nasal breathing of newborns during and after feeding, as they must forcefully close their mouth to suck properly.[20] It is considered a nutritive sucking habit, since it feeds the infant. Contrarily, introducing bottle-feeding early in the infant's life triggers rapid satiety and does not involve as much of a sucking effort. This leads the newborn to satisfy its suction desire through non-nutritive suction – sucking on objects that do not provide food, such as fingers, pacifiers or toys.[21,22]

Sucking fingers and/or pacifiers are the most common non-nutritive sucking habits among infants. The early introduction of a pacifier and/or a feeding bottle may confuse the newborn's sucking reflex, resulting in premature weaning. Non-nutritive habits can cause different alterations of dental occlusion depending on their frequency, intensity and duration. Dental occlusion alterations are developmental disorders that occur in the dentofacial structure, comprising the jaw, tongue and facial muscles.[5] The baby's sucking during breastfeeding stimulates the balance between the internal and external restraining forces of the facial musculature. This allows the appropriate development of the stomatognathic system and the adequate growth of the craniofacial complex, which can play an important role in preventing occlusal disorders in the child's primary dentition.[3,5,7,23–25]

Breastfeeding has been reported to protect against occlusal alterations in the primary dentition. Some studies report protection against occlusal alterations after 6 months of breastfeeding, and other emphasize the need for longer periods of breastfeeding for greater benefits.[3,5,15,26,27] However, only 40% of infants worldwide are exclusively breastfed for the first 6 months.[27] This average reduces to 37% in low- and middle-income countries.[28] It is known that when a baby is weaned after receiving breastfeeding, if a bottle is offered as a substitute, and used for a long time and at high frequency, it can overshadow the benefits of breastfeeding. Well-designed studies make it possible to identify whether prolonged breastfeeding is a protective factor against the appearance of harmful habits, or transversal and vertical occlusal alternations. They allow us to know the consistency of the results in a specific association. Given this context, the main purpose of the present study was to determine the association between the duration of breastfeeding and the development of non-nutritive habits, and transversal and vertical occlusal alterations in children aged 2–5 years from Lima, Peru.

Material and methods

This study was approved by the Ethics Committee at the School of Dentistry of the Scientific University of the South (Universidad Científica del Sur), Lima, Peru (No. of approval: 000444). The parents or legal guardians of the children signed the informed consent forms prior to participation in the study.

The sample was composed of 155 children aged 2–5 years, of both genders, from 3 public schools in Lima, Peru. They were distributed into 2 groups based on the duration of breastfeeding: group A (n = 50) included children that had been breastfed for 0–6 months; and group B (n = 105) included children that had been breastfed for more than 6 months and up to 1 year.

Children with systemic diseases, uncooperative, with morphological alterations, not breastfed from birth, with tooth decay injuries that covered more than 50% of the tooth surface, or whose parents did not provide consent to their participation were excluded.

Non-nutritive habits, and transversal and vertical occlusal alterations were clinically evaluated by 2 previously trained and calibrated examiners.

Evaluation of non-nutritive habits

Digital sucking was evaluated through the presence or absence of a digital callus on at least 1 finger, validated with the child's caregiver's response to the survey, and the presence of some type of characteristic related to sucking in the oral cavity, such as disturbances in the arch form, wrinkled, chapped or blistered fingers, ulceration or corn formation, etc.

Labial sucking was determined through clinical observations. It was considered as present in the children who unconsciously sucked their lips during the evaluation process, presenting a constantly moisturized lower lip. The observations were corroborated by the child's caregiver's response to the survey.

To assess atypical swallowing, children were asked to swallow their saliva. Then, they were asked to eat crackers. Thus, liquid and food swallowing were observed. The following criteria were checked: effortless lip sealing; lingual interposition; movement of the head or other parts of the body; tension of the mentalis muscle; tension of the orbicularis oris muscle; leakage of food; and noise. Children were considered to swallow atypically when they met at least 3 of these criteria.

Oral breathing was assessed through the child's caregiver's response to the survey on whether the child snored or drooled at night. Furthermore, 7 clinical criteria were also taken into account: presence of eye shiners; characteristics of the nostrils; dry lips; underdeveloped maxillary bone due to the lack of maxillary sinus development; lip incompetence; palate type; and anterior open bite, as well as a breathing test that consisted in positioning a mouth mirror over the child's nostrils. Oral breathing was considered present when a child met at least 5 of these criteria.

Also, the children's parents or caregivers were asked to fill in a questionnaire; it included questions about the child and the mother, the type and duration of breastfeeding, the use of a bottle and the duration of bottle-feeding, the presence of habits such as digital or labial sucking, atypical swallowing, the type of breathing (either nasal or oral), snoring, and drooling.

Evaluation of transversal occlusal alterations

Posterior crossbite (PCB) was clinically evaluated; it is considered present when the vestibular surfaces of the upper molars fit behind the vestibular surfaces of the lower molars. It can be unilateral if no midline shift exists, but in general, PCB is bilateral. Likewise, 2 types were considered for the diagnosis of a complete PCB – buccal, when the upper jaw completely covered the lower jaw, or lingual, when the lower jaw completely covered the upper jaw.

Evaluation of vertical occlusal alterations

Open bite was clinically evaluated by measuring the distance between the incisal edges of the maxillary and mandibular central incisors. The end-to-end incisor relationship or the negative overbite condition were considered open bite.

Deep bite was clinically evaluated by measuring the coverage percentage of the upper central incisor on the vestibular surface of the lower central incisor; deep bite was considered present when the coverage was more than 30%.

Study error

All evaluations were performed again for all variables by the same examiner after a 10-day interval. The intra-examiner concordance was evaluated with the kappa coefficient, achieving values greater than 0.9 for all the outcome variables.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, v. 25.0 (IBM Corp., Armonk, USA). The χ^2 test was used to analyze the possible associations between breastfeeding duration and the presence of non-nutritive habits or occlusal alterations. Subsequently, logistic regression analysis was performed to identify the presence of risk factors with regard to the outcome variables. The duration of breastfeeding (in months), the duration of bottle use (in months), gender, age, the parents' educational level, and the number of children in the family were considered predictor variables. The overfit method was used. At first, a primary regression considering all predictor variables was performed. Then, a final regression selecting only the variables with a p-value <0.25 was performed. The significance level was set at $p < 0.05$ for all tests.

Results

The gender distribution is shown in Table 1. Breastfeeding duration was significantly associated with oral breathing ($p = 0.048$); the possible oral breathing was lesser in children that had been breastfed for more than 6 months (Table 2). No other statistically significant associations were found (Tables 1–3).

The logistic regressions showed that the use of a bottle for more than 24 months significantly influenced the appearance of non-nutritive habits (*OR* (odds ratio) = 3.55; 95% *CI* (confidence interval): 1.20–10.55; $p = 0.022$) (Table 4). Breastfeeding duration (*OR* = 0.84; 95% *CI*: 0.74–0.96; $p = 0.011$) and gender (*OR* = 0.22; 95% *CI*: 0.07–0.69; $p = 0.009$) significantly affected the development of oral breathing (Table 5). The probability of the occurrence of the oral breathing habit was greater in the case of shorter breastfeeding duration and in girls (Table 5).

Regarding occlusal alterations, the use of a bottle for more than 24 months significantly influenced the appearance of anterior open bite (*OR* = 12.12; 95% *CI*: 1.16–126.31; $p = 0.037$). Furthermore, boys were found to be less likely to have anterior open bite (*OR* = 0.27; 95% *CI*: 0.08–0.99; $p = 0.048$). Older preschool children appeared to be less likely to present PCB (*OR* = 0.10; 95% *CI*: 0.02–0.54; $p = 0.007$). Finally, the youngest children in the family presented a decreased likelihood of having deep bite (*OR* = 0.45; 95% *CI*: 0.26–0.77; $p = 0.003$) (Table 6).

Discussion

There are few studies regarding the minimum duration of breastfeeding that protects newborns against the development of non-nutritive habits or dental occlusion alterations,

Table 1. Gender distribution in both evaluated groups

Group	Gender		Total	p-value
	M	F		
Group A	27 (54.0)	23 (46.0)	50 (100)	0.391
Group B	48 (45.7)	57 (54.3)	105 (100)	
Total	75 (48.4)	80 (51.6)	155 (100)	

Data presented as number (percentage) (*n* (%)). M – male; F – female. Fisher's exact test.

Table 2. Associations between the duration of breastfeeding and the appearance of oral habits

Oral habit		Group A $n = 50$	Group B $n = 105$	Total $N = 155$	p-value
Appearance of oral habits	absent	23 (46.0)	57 (54.3)	80 (51.6)	0.391
	present	27 (54.0)	48 (45.7)	75 (48.4)	
	total	50 (100)	105 (100)	155 (100)	
Digital sucking	absent	44 (88.0)	98 (93.3)	142 (91.6)	0.352
	present	6 (12.0)	7 (6.7)	13 (8.4)	
	total	50 (100)	105 (100)	155 (100)	
Labial sucking	absent	36 (72.0)	74 (70.5)	110 (71.0)	1.000
	present	14 (28.0)	31 (29.5)	45 (29.0)	
	total	50 (100)	105 (100)	155 (100)	
Atypical swallowing	absent	28 (56.0)	63 (60.0)	91 (58.7)	0.728
	present	22 (44.0)	42 (40.0)	64 (41.3)	
	total	50 (100)	105 (100)	155 (100)	
Oral breathing	absent	35 (70.0)	88 (83.8)	123 (79.4)	0.048*
	present	15 (30.0)	17 (16.2)	32 (20.6)	
	total	50 (100)	105 (100)	155 (100)	
Snoring	absent	32 (64.0)	81 (77.1)	113 (72.9)	0.121
	present	18 (36.0)	24 (22.9)	42 (27.1)	
	total	50 (100)	105 (100)	155 (100)	
Drooling	absent	28 (56.0)	60 (57.1)	88 (56.8)	1.000
	present	22 (44.0)	45 (42.9)	67 (43.2)	
	total	50 (100)	105 (100)	155 (100)	

Data presented as *n* (%). * statistically significant (Fisher's exact test).

Table 3. Associations between the duration of breastfeeding and the appearance of posterior crossbite (PCB), open bite and deep bite

Bite		Group A $n = 50$	Group B $n = 105$	Total $N = 155$	p-value
PCB	absent	47 (94.0)	101 (96.2)	148 (95.5)	0.682
	present	3 (6.0)	4 (3.8)	7 (4.5)	
	total	50 (100)	105 (100)	155 (100)	
Open bite	absent	42 (84.0)	93 (88.6)	135 (87.1)	0.290
	present	8 (16.0)	12 (11.4)	20 (12.9)	
	total	50 (100)	105 (100)	155 (100)	
Deep bite	absent	20 (40.0)	39 (37.1)	59 (38.1)	0.728
	present	30 (60.0)	66 (62.9)	96 (61.9)	
	total	50 (100)	105 (100)	155 (100)	

Data presented as *n* (%). Fisher's exact test.

Table 4. Binary logistic regression to identify the predictor variables for the appearance of oral habits

Predictor variable		Appearance of oral habits			
		OR	95% CI lower limit	95% CI upper limit	p-value
Duration of breastfeeding [months]		1.02	0.97	1.08	0.424
Duration of bottle use [months]	<12	–	–	–	0.069
	13–24	1.88	0.75	4.75	0.180
	>24	3.55	1.20	10.55	0.022*
Gender	F	–	–	–	–
	M	1.11	0.51	2.40	0.790
Age [years]		0.91	0.61	1.34	0.628
Number of children in the family		0.78	0.52	1.18	0.241

OR – odds ratio; *CI* – confidence interval; * statistically significant. $R^2 = 7.2\%$ (Cox–Snell).

Table 5. Binary logistic regression to identify the predictor variables for the appearance of oral breathing

Predictor variable		Oral breathing			
		OR	95% CI lower limit	95% CI upper limit	p-value
Duration of breastfeeding [months]		0.84	0.74	0.96	0.011*
Duration of bottle use [months]	<12	–	–	–	0.386
	13–24	1.62	0.35	7.47	0.538
	>24	2.75	0.62	12.24	0.185
Gender	F	–	–	–	–
	M	0.22	0.07	0.69	0.009*
Age [years]		–	–	–	–
Parents' educational level	secondary education	–	–	–	–
	college graduate	0.92	0.22	3.92	0.915
Number of children in the family		0.81	0.43	1.52	0.505

* statistically significant. $R^2 = 24.2\%$ (Cox–Snell).

and they report varied results.[28–30] Previous systematic reviews generally found a protective effect of breastfeeding on allergic outcomes, although many studies had methodological limitations.[20] Although breastfeeding is reported to protect against lower respiratory tract infections during infancy, such protection has not been clearly demonstrated for asthma.[26] Children who are breastfed generally adopt nasal breathing due to the hermetic mouth seal during sucking, thus reducing their susceptibility to allergies; consequently, they develop fewer non-nutritive habits and fewer occlusal alterations.[19] To confirm this relationship, more studies are needed. In this regard, this study sought

Table 6. Binary logistic regression to identify the predictor variables for the appearance of posterior crossbite (PCB), open bite and deep bite

Predictor variable		PCB				Open bite				Deep bite			
		OR	95% *CI* lower limit	95% *CI* upper limit	*p*-value	*OR*	95% *CI* lower limit	95% *CI* upper limit	*p*-value	*OR*	95% *CI* lower limit	95% *CI* upper limit	*p*-value
Duration of breastfeeding [months]		0.92	0.73	1.16	0.479	0.93	0.81	1.07	0.294	1.06	0.99	1.14	0.107
Duration of bottle use [months]	<12	–	–	–	0.231	–	–	–	0.105	–	–	–	0.347
	13–24	3.55	0.20	64.30	0.392	6.73	0.61	73.96	0.119	0.47	0.16	1.40	0.175
	>24	0.54	0.02	15.48	0.716	12.12	1.16	126.31	0.037*	0.49	0.15	1.61	0.238
Gender	F	–	–	–	–	–	–	–	–	–	–	–	–
	M	0.26	0.03	2.59	0.249	0.27	0.08	0.99	0.048*	1.88	0.81	4.38	0.144
Age [years]		0.10	0.02	0.54	0.007*	0.62	0.30	1.27	0.189	1.00	0.64	1.57	0.999
Parents' educational level	secondary education	–	–	–	–	–	–	–	–	–	–	–	–
	college graduate	0.47	0.03	8.68	0.614	4.58	0.60	34.90	0.142	1.60	0.55	4.65	0.389
Number of children in the family		0.40	0.07	2.20	0.291	1.97	0.96	4.02	0.063	0.45	0.26	0.77	0.003*

* statistically significant. R^2 = 15.8% for PCB; R^2 = 23.7% for open bite; R^2 = 16.6% for deep bite (Cox–Snell).

to determine the association between breastfeeding duration, the development of non-nutritive habits, and transversal and vertical occlusal alterations in children aged 2–5 years from Lima, Peru. The aim was to demonstrate whether prolonged exclusive breastfeeding for more than 6 months acts as a protective factor against the development of non-nutritive sucking habits or occlusal alterations. For this purpose, data collected through questionnaires and clinical assessments was analyzed.

The findings of this study reinforce the notion that a longer breastfeeding period may lead to a decrease in the occurrence of some non-nutritive habits, specifically oral breathing, and more consistency about this association is now available. The group that had been breastfed for more than 6 months had a significantly smaller percentage of children with oral breathing (16.2%) (Table 2). In addition, the regression results showed that breastfeeding duration was a protective factor against this habit (Table 5). To understand how breastfeeding duration works as a protective factor against oral breathing, it should be noted that during breastfeeding, babies adopt nasal breathing due to the hermetic mouth seal which occurs during suction, reinforcing nasal respiration during feeding. During suction, children open, protrude, close, and retrude their jaws, developing the entire muscular system of masseter, temporal and pterygoid muscles. Additionally, the temporomandibular joint is stimulated during breastfeeding and the anteroposterior growth of the mandibular body is expected. Conversely, during bottle-feeding, the tongue is positioned lower and further back, and the lips are separated; therefore, the tongue propulsion is weaker. Furthermore, pterygoid muscles and masseters are used less, because, due to the lingual depression at the time the baby carries out the passage of the food, they cannot be synchronized with respiration, thus increasing the probability of oral breathing.[31,32] Breastfeeding promotes nasal respiration due to suction, and this results in the adequate craniofacial development and prevents respiratory infections.[33,34]

Breastfeeding is promoted for its multiple benefits, both nutritional and psychological. There is consensus among different institutions and organizations that in order to guarantee its benefits, breastfeeding should be the exclusive source of feeding for a baby for 6 months or more in low- and middle-income countries.[7,14,22] Our findings reinforce these recommendations and the role of breastfeeding with regard to oral breathing.

Furthermore, exclusive breastfeeding is strongly and inversely associated with the frequency, intensity and duration of the use of a bottle, which can lead to occlusal alterations. The use of baby bottles is extremely popular for several reasons. One very important reason is that many mothers go to work and cannot breastfeed their children. The prolonged use of a bottle could be associated with the lack of knowledge of parents about its harmful effects and how it can influence occlusal alterations in children. We found that the use of a bottle for more than 24 months significantly influenced the appearance of open bite (Table 6). Furthermore, we found that the duration of bottle-feeding was a risk factor with regard to the appearance of oral habits (Table 4). Hence, bottle use should be avoided. Contrarily, the duration of breastfeeding was not related to the occurrence of transversal or vertical occlusal alterations (Table 6). However, we identified some factors that affected the outcome variables, for instance, boys were found to be less likely to have anterior open bite (*OR* = 0.27; 95% *CI*: 0.08–0.99; p = 0.048), older preschool children appeared to be less likely to present PCB (*OR* = 0.10; 95% *CI*: 0.02–0.54; p = 0.007) and the youngest children in the family presented a decreased likelihood of having deep bite (*OR* = 0.45; 95% *CI*: 0.26–0.77; $p = 0.003$) (Table 6). Although these predictor variables showed an influence on the appearance of various types of malocclusion, we must remember that they can be accompanied by several environmental factors, and that a genetic factor should always be taken into account.

As this was a meticulous study, we found some challenges when carrying it out, especially when collecting essential information from the parents and/or guardians. Further research with larger populations is needed for more general conclusions. Even though both groups had different sample sizes and the size of group A was considerable (50 participants), so the results should not have been affected, yet other studies with different samples might corroborate the results of the present study.

Our findings are relevant to the promotion of public health, pediatric dentistry and orthodontics. Clinicians should be aware of the benefits of prolonged exclusive breastfeeding. Therefore, they can educate laypeople on the correct practice of exclusive breastfeeding to obtain all of its benefits. Finally, the results of this study allow us to conclude that preschool children who have prolonged breastfeeding have a lower probability of presenting oral breathing. In addition, the prolonged use of a baby bottle can produce occlusal alterations and lead to the presence of anterior open bite in children.

Conclusions

Breastfeeding duration of 6–12 months was shown to be a protective factor in preventing the appearance of oral breathing. Posterior crossbite, open bite and deep bite seemed not to be influenced by breastfeeding duration. However, the use of a bottle for more than 24 months significantly influenced the appearance of anterior open bite.

Ethics approval and consent to participate

This study was approved by the Ethics Committee at the School of Dentistry of the Scientific University of the South (Universidad Científica del Sur), Lima, Peru (No. of approval: 000444). The parents or legal guardians of the children signed the informed consent forms prior to participation in 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.

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Original paper

Paraoxonase-1, a novel link between periodontitis and ischemic heart disease: A case–control study

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Abstract

Background. Periodontitis is a chronic inflammatory disease and might be a potential risk factor for ischemic heart disease (IHD). However, the link between periodontitis and atherosclerosis is not yet fully understood. Paraoxonase-1 (PON-1) is a new biomarker representing both anti-atherosclerotic and antioxidant activity, which also acts against dental biofilm formation and periodontitis. The possible contributing role of PON-1 in the relationship between periodontitis and atherosclerosis has not been studied to date.

Objectives. The aim of the present study was to investigate the serum level of PON-1 with regard to the periodontal status in IHD patients.

Material and methods. In this case–control study, 67 patients with IHD underwent a periodontal examination and were accordingly allocated to one of the 2 study groups: the case group with chronic periodontitis ($n = 36$); or the control group with a healthy periodontium ($n = 31$). Serum PON-1 activity was measured by means of colorimetric analysis.

Results. There were no significant differences between the groups in terms of demographic data, cardiac risk factors, initial biochemical test results, cardiac pump function, and the number of grafted vessels. The activity of PON-1 in cardiac patients suffering from periodontitis was significantly lower than in cardiac patients with a healthy periodontal status (53.01 ±7.53 U/mL and 59.11 ±9.95 U/mL, respectively; $p = 0.007$).

Conclusions. This finding suggests that the combination of IHD and periodontitis is associated with lower PON-1 activity. Further studies might be required to assess the possible role of periodontal treatment in increasing PON-1 activity and reducing IHD severity.

Keywords: chronic periodontitis, cardiovascular disease, coronary artery bypass, paraoxonase-1

Introduction

Periodontitis is a chronic disease characterized by inflammatory[1,2] and immune reactions.[3] Periodontitis and ischemic heart disease (IHD) have a variety of common risk factors, such as diabetes mellitus, old age, low socioeconomic status, and obesity.[4–6] Both are non-contagious maladies and are expressed by high prevalence on a global scale. Several chronic infectious diseases,[7] as well as inflammatory and immunity disorders, are important risk factors for both. Periodontitis and IHD may represent common pathophysiology.[5,6,8–11] This can be confirmed by identifying the susceptible genes for both diseases.

Epidemiologic evidence shows that periodontitis leads to a higher risk of atherosclerotic cardiovascular disease in the future.[1,12] Untreated periodontal disease can result in a severe systemic inflammatory state.[12] Numerous studies have indicated that increased serum levels of endotoxins (lipopolysaccharides) and inflammatory cytokines, such as C-reactive protein (CRP), thromboxane A2 (TxA2), interleukin-1 beta (IL-1β), prostaglandin E2 (PGE2), and tumor necrosis factor-alpha (TNF-α), are associated with periodontitis.[2,13] These markers are also shown to be capable of initiating and exacerbating atherogenic and thromboembolic events.[2,12–14] The effect of periodontal disease on IHD seems to consist in the change in oral microbiota, and the direct or indirect influence of systemic inflammation. Therefore, periodontitis appears to be a modifiable and nonconventional risk factor for IHD.

Paraoxonase-1 (PON-1) is a 43 kDa polypeptide containing 355 amino acids.[15,16] It is a polymorphic protective enzyme synthesized in the liver. Paraoxonase-1 binds to high-density lipoprotein particles, and it seems to be an important factor in anti-atherosclerotic, anti-inflammatory and antioxidative processes. Moreover, it acts against bacterial biofilm formation. Serum PON-1 activity is significantly lower in patients suffering from cardiovascular, liver, diabetic, and renal diseases, as well as obesity and cancer. This enzyme is suggested to be considered as a new predictive biomarker and a surrogate endpoint. There are limited studies on the importance of PON-1 in periodontal patients.

To our knowledge, the possible contributing role of PON-1 in the relationship between periodontitis and atherosclerosis has not been studied to date. The purpose of the present study was to evaluate serum PON-1 activity with regard to the periodontal status in patients with documented IHD who underwent coronary artery bypass grafting (CABG) surgery.

Material and methods

This case–control study included IHD patients who were scheduled for elective CABG in a tertiary university hospital in Tehran, Iran, from June 2020 to June 2021. The study protocol was approved by the Ethics Committee at the Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.DRC.REC.1398.225). Documented informed consent was obtained from all the involved participants. The study was reported in accordance with the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) statement.

The inclusion criteria were IHD patients undergoing elective isolated CABG surgery with at least 15 teeth per individual. Our exclusion criteria were as follows: 1) having a history of smoking or opioid use; 2) patients with systemic diseases, such as diabetes, obesity, rheumatoid arthritis, and systemic infections; 3) patients who had been taking antibiotics, anti-inflammatory (except aspirin) and steroid medications within the last 6 months; 4) individuals who had undergone periodontal surgery, or scaling and root planing within the last 6 months; 5) candidates for an urgent surgery; 6) patients who had a history of infarction within the last 2 weeks; 7) patients with other pathophysiological conditions of the heart, liver or kidneys; and 8) patients with stage II periodontitis.

For all individuals, a periodontal examination was performed prior to the surgery by one dental student who was trained within a standard educational program. The gingival index (GI), bleeding on probing (BOP), the probing pocket depth (PPD), and the clinical attachment level (CAL) were assessed in 6 areas of the teeth (mesiobuccal, midbuccal, distobuccal, mesiolingual/palatal, midlingual/palatal, and distolingual/palatal). The examined teeth were maxillary central incisors, maxillary first premolars, maxillary first molars, mandibular central incisors, mandibular first premolars, and mandibular first molars. The armamentarium that was used for the periodontal examination were the Williams periodontal probe (Hu-Friedy, Chicago, USA), a dental mirror and dental explorer No. 33 (Hu-Friedy).

According to the Löe and Silness classification, GI is an indicator of gingival tissue condition. This index considers the quality of gingiva (the severity of lesions) and the affected areas. Therefore, it does not refer to the pocket depth, bone loss and other changes in the quantity of periodontal tissues. The PPD is defined as the distance between the gingival margin and the base of the periodontal pocket. The CAL is the distance between the cementoenamel junction and the base of the periodontal pocket.

The patients were subsequently allocated to the case and control groups with regard to their periodontal status. Periodontal disease was defined based on the consensus report of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions.[1] The IHD patients with stage III or IV were included in the case group. The control group consisted of individuals who had been diagnosed with either a healthy periodontium or stage I periodontitis.

Demographic information about this population, the patients' cardiac condition, and their results of routine laboratory tests and the periodontal examination were recorded. The baseline inflammatory status was established by measuring the erythrocyte sedimentation rate (ESR), the white blood cell count (WBC), the red blood cell distribution width (RDW), and the mean platelet volume (MPV).

All patients underwent identical cardiac treatment, regardless of the result of the periodontal examination. The investigators, the patients and the medical team were blind to the periodontal allocation.

Table 1. Demographic and medical data of the study participants ($N = 67$)

Characteristic	IHD patients with periodontitis $n = 36$	IHD patients with a healthy periodontium $n = 31$	*p*-value
Age [years]	55.50 ±10.00	58.32 ±8.47	0.160
Gender (F/M)	22 (61.11)	16 (51.61)	0.460
History of hyperlipidemia (+/–)	17 (47.22)	19 (61.29)	0.250
History of hypertension (+/–)	19 (52.78)	20 (64.52)	0.330
Triglyceride [mg/dL]	150.24 ±68.54	164.18 ±70.32	0.440
Cholesterol [mg/dL]	144.84 ±46.13	158.63 ±38.87	0.240
LDL [mg/dL]	84.26 ±29.72	86.28 ±31.56	0.830
HDL [mg/dL]	39.37 ±13.42	41.33 ±17.89	0.710
Serum vitamin D [ng/dL]	21.67 ±11.34	24.20 ±17.77	0.520
Basic albumin amount [g/dL]	3.79 ±0.41	3.72 ±0.44	0.940
ESR [mm/h]	17.13 ±12.70	18.19 ±14.62	0.750
Basic WBC [$\times10^3$ µL]	7.17± 1.70	8.23 ±2.76	0.370
PLT [$\times10^3$ µL]	270.64 ±83.22	231.68 ±60.89	0.030*
RDW [%]	13.30 ±1.31	13.49 ±0.93	0.230
MPV [fL]	9.58 ±1.40	10.11 ±1.42	0.060
Number of grafted vessels	3.33 ±0.74	3.50 ±0.71	0.250
EF [%]	45.30 ±11.80	44.29 ±10.00	0.590
PON-1 activity (U/mL)	53.01 ±7.53	59.11 ±9.95	0.007*

Data presented as mean ± standard deviation ($M \pm SD$) or as number (percentage) (n (%)). IHD – ischemic heart disease; F – female; M – male; LDL – low-density lipoprotein; HDL – high-density lipoprotein; ESR – erythrocyte sedimentation rate; WBC – white blood cell count; PLT – platelet count; RDW – red blood cell distribution width; MPV – mean platelet volume; EF – ejection fraction; PON-1 – paraoxonase-1; * statistically significant.

Measuring PON-1 activity

Fasting blood samples were collected from all patients into simple tubes prior to the operation and given enough time to make a clot. The tubes were subsequently centrifuged at 1,000 rpm for 3–5 min to form a clear serum, which was stored at −70°C. Serum PON-1 activity was measured using a colorimetric assay kit (ZellBio, Lonsee, Germany) according to the manufacturer's instructions.

Statistical analysis

Quantitative and qualitative variables were expressed as mean and standard deviation ($M \pm SD$), and as number and percentage (n (%)), respectively. Continuous and categorical variables were compared between the groups using Student's t test and the χ^2 test, respectively. Statistical analysis was performed using the Stata software, v. 13 (StataCorp, College Station, USA), and $p < 0.05$ was considered statistically significant.

Results

Our study population consisted of 67 patients with indications for CABG surgery, who were enrolled between June 2020 and June 2021 in a tertiary university hospital. Subsequently, the IHD patients were allocated to either the group with a healthy periodontium ($n = 31$) or the group with chronic periodontitis ($n = 36$). Table 1 shows that the patients showed no remarkable variations in the demographic data, cardiac risk factors, the initial biochemical tests, IHD severity, and the number of stenotic coronary arteries. The findings of the periodontal examinations in IHD patients are presented in Table 2.

The baseline inflammatory status in terms of ESR, WBC, RDW, and MPV was comparable between the groups. The platelet count (PLT) was significantly different between the 2 groups ($p = 0.030$). Although it might not have any clinical significance in coagulation activity,

Table 2. Periodontal examination data of the study participants ($N = 67$)

Periodontal index		IHD patients with periodontitis $n = 36$	IHD patients with a healthy periodontium $n = 31$	*p*-value
GI (0–3)	0	0 (0)	31 (100)	<0.001*
	2	36 (100)	0 (0)	
BOP [%]		36 (100)	0 (0)	<0.001*
PPD [mm]		5.8 ±0.9	1.4 ±0.8	<0.001*
CAL [mm]		5.1 ±1.4	2.4 ±1.8	<0.001*

Data presented as $M \pm SD$ or as n (%). GI – gingival index; BOP – bleeding on probing; PPD – probing pocket depth; CAL – clinical attachment level; * statistically significant.

it can represent a higher level of acute-phase proteins in IHD patients with periodontitis.

The activity of PON-1 was significantly lower in patients suffering from both cardiac and periodontal diseases (stage III or IV) in comparison with cardiac patients who had a healthy periodontium or stage I periodontitis ($p = 0.007$) (Table 1).

Discussion

The present study showed that PON-1 activity was significantly lower in IHD patients suffering from stage III and IV periodontitis in comparison with IHD patients with a healthy periodontium or stage I periodontitis.

Periodontitis is a chronic disease with a high prevalence of 45–50%.[1,8,9,12] A severe form of the disease affects more than 10% of the worldwide population[17] and is considered to be the 6th most common disease among humans.[18] Periodontitis is initiated with the inflammation of gingiva induced by bacteria present in dental plaque. Randomized control trials report that patients with periodontitis experience higher levels of inflammatory mediators in their serum, which significantly decrease following periodontal treatment.[7,14,18] Frequent bacteremia allows pathogens to enter the systemic blood circulation, activate the inflammatory cascades, and potentially cause endothelial and cardiac dysfunction. Therefore, systemic inflammation might represent a biological link between periodontitis and cardiovascular diseases.[4–7,14,19]

Gram-negative bacteria use homoserine lactones (HSLs) as signals of quorum sensing to enhance their biofilm. Recent evidence indicates that PON-1 might play an essential role in the protection against the formation of bacterial biofilm.[20–22] An experimental study showed that a reduced level of PON-1 led to a decrease in HSL hydrolysis, which induced dental biofilm formation and periodontitis.[21] In addition, an in vitro study suggested that PON-1 played an important role in the osteoblastic differentiation of periodontal ligaments.[23] Several clinical studies showed that the acute-phase response induced by endotoxins and lipopolysaccharides, as well as an increased release of inflammatory cytokines can decrease PON-1 activity in serum.[22,24]

Moreover, PON-1 has a protective function against atherosclerosis.[16] Preclinical studies showed that PON-1 inhibited the oxidation of low-density lipoprotein (LDL).[16,23] Oxidized LDL is plays an essential role in foam cell formation and plaque development.[7] Sufficient levels and activity of PON-1 can decrease oxidative stress, lipid peroxidation and the risk of IHD.[1,16] In the same manner, low PON-1 activity and the insufficiency of the enzyme in plasma have been shown to be related to high susceptibility to IHD.[22,24]

Since 1980, some evidence has been published, explaining that the dental health of patients with myocardial infarction was significantly poorer in comparison with the control group. Until October 2018, there was no published information about the effect of periodontal treatment on the initial intervention for acute myocardial infarction. The Periodontitis and Vascular Events (PAVE) study was the only published pilot study testing the effect of periodontal treatment on the secondary prevention of cardiovascular outcomes, and it concluded that periodontal treatment did not lead to a considerable decrease in cardiovascular events.[7] However, it must be noticed that due to the small number of patients, this pilot study was not strong enough to evaluate the influence of periodontitis on cardiovascular events. Our study showed lower PON-1 activity in IHD patients with periodontitis in comparison with IHD patients who had a normal periodontal status. Nevertheless, the clinical significance of the activity of PON-1 as a protective agent against both IHD and dental biofilm formation requires further studies.

On the other hand, in a cohort study conducted on 8,999 patients with periodontitis who had received periodontal treatment (nonsurgical or surgical, if necessary) and had been followed up according to the treatment protocols from 1979 to 2012, subjects who showed a weak response to periodontal treatment presented with more acute IHD as compared to those who appropriately responded to the treatment.[1] This suggests that successful periodontal treatment may reduce the incidence of acute IHD. Previous evidence showed a higher level of CRP in patients who had both IHD and periodontitis as compared to patients who had either periodontal disease or heart problems.[18] The effect of periodontal treatment is related to a considerable decrease in the CRP level and enhancement in the cardiovascular health criterion.[1] Confirming our findings, PON-1 has a direct effect on the prevention of LDL oxidation and plaque formation, and its association with periodontitis might be meaningful. Noack et al. showed that diabetic patients had an increased risk of periodontitis and lower PON-1 activity as compared to pre-diabetic patients.[24] It may corroborate our finding that the activity of PON-1 as an anti-inflammatory and anti-atherosclerotic marker was reduced in IHD patients with periodontitis. Diminished PON-1 activity may exacerbate the inflammation-induced atherosclerotic process in the heart.

Examining the patients by a dental student is a limitation of the study. Moreover, the sample size under examination was small. Nevertheless, the patients were from a homogenous population with regard to the cardiac risk factors and the severity of coronary artery disease. To the best of our knowledge, there is no similar study assessing the role of PON-1 activity in periodontal and cardiac conditions simultaneously. Hence, the measurement of PON-1 activity might be considered a new biomarker in the evaluation of periodontal and cardiac treatment.

Conclusions

The protective function of PON-1 might be important for the tissues frequently affected by severe oxidative stress and bacterial infections, such as the periodontium. Paraoxonase-1 as a new biomarker may introduce a novel link between periodontitis and atherosclerosis. Patients with IHD are recommended to maintain a routine periodontal check-up as part of their management to detect changes in the inflammatory status of the periodontium. Further studies are suggested to assess an increase in PON-1 activity following periodontal treatment in IHD patients and its effect on clinical practice.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee at the Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.DRC.REC.1398.225). Documented informed consent was obtained from all the involved 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.

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Original paper

Assessment of myocardial strain in hypertensive patients with periodontitis

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Background. Left ventricular (LV) relaxation is affected by hypertension. The inflammatory mediators produced in response to systemic inflammation, such as in periodontal disease, may also alter ventricular mechanics and the existing ventricular dysfunction. Thus, the systemic inflammatory burden which occurs in response to chronic periodontitis may alter myocardial activity.

Objectives. The current study aimed to assess the myocardial strain among controlled hypertensive patients with periodontitis by using two-dimensional (2D) echocardiography.

Material and methods. The study involved 150 controlled hypertensive patients, equally divided into group A (without periodontitis) and group B (with periodontitis). The cardiac strain was measured with 2D echocardiography and represented as global longitudinal strain (GLS), while the periodontal inflamed surface area (PISA) score quantified the systemic inflammatory burden experienced by these individuals due to chronic periodontitis.

Results. In the multiple linear regression model, the adjusted R^2 for group B indicated that 88% of the variation in GLS was due to the independent variable (PISA). Thus, with every one-unit rise in PISA, there was a mild alteration in GLS of 7.54×10^{-5}. A scatter plot depicted a positive correlation between PISA and GLS.

Conclusions. Within the limitations of the study, it can be concluded that an increase in the PISA score may cause mild alterations in the GLS score, which could indicate the possible influence of periodontitis on myocardial activity.

Keywords: cardiovascular diseases, inflammation, periodontal pocket, left ventricle, cardiac imaging technique

Introduction

Periodontitis is an inflammatory disease of the structures supporting the teeth, affecting 10–15% of the global population, which poses a public health challenge. This inflammation brings about a systemic inflammatory burden and influences the existing systemic conditions, such as diabetes mellitus, coronary heart disease, stroke, etc.[1,2] An epidemiological study by the American Academy of Periodontology and the American Heart Association states that in some patients, biological factors, such as chronic inflammation, might independently link periodontal disease to atherosclerosis and the development or progression of cardiovascular disease.[3] This coronary atherosclerosis progressively leads to the ischemia of the myocardium, thereby leading to cardiovascular complications.[3]

The myocardium plays a pivotal role in cardiac function; any myocardial malperformance may impact the overall health. Two-dimensional (2D) echocardiography has provided a new perspective in understanding myocardial mechanics, helping us to identify early left ventricular (LV) dysfunction,[4] expressed as global longitudinal strain (GLS).[5] The GLS analysis has gained popularity, as it is a feasible and non-invasive diagnostic modality to assess cardiac mechanics, and it can detect subclinical alterations in ventricular function.[6] Longitudinal strain represents the change in the length of the myocardium along the long axis of the left ventricle (LV) (from the base to the apex). It represents the rate at which the deformation progresses. The deformation refers to the myocardium changing shape and dimensions during the cardiac cycle, i.e., during systole and diastole.[7] The strain rate is the quantum of deformation per unit of time. It reflects regional myocardial velocity in tissue Doppler imaging. Strain rate imaging has several hues, e.g., green denotes no strain rate, orange-red indicates a negative strain rate and blue indicates a positive strain rate.[8]

Left ventricular dysfunction occurs early in most cardiac diseases and frequently goes unnoticed, since it is a preclinical condition where LV fails to fill an adequate end-diastolic volume at an acceptable pressure, which may lead to heart failure (HF).[9] Left ventricular function (LVF) is assessed using the ejection fraction (EF)[10]; however, recently, GLS has gained popularity, as it can track early alterations in LVF. Therefore, GLS can be used as an early indicator of cardiac dysfunction, and can help recognize individuals in the general population who are at a notably greater risk of cardiovascular morbidity and death. The periodontal inflamed surface area (PISA), measured in square millimeters, refers to the surface area of the bleeding pocket epithelium. The PISA parameter quantifies the amount of inflamed periodontal tissues in each patient, and is comparatively more accurate than any other classification currently in use.

Hypertension affects LF relaxation, thereby reducing LV compliance due to LV hypertrophy.[10] Furthermore, the endotoxins and inflammatory markers produced in response to systemic inflammation may alter ventricular mechanics and the existing ventricular dysfunction.[11] Among hypertensive patients with periodontitis, greater LV mass and LV mass index are observed, which indicates structural changes rather than functional changes in the ventricles.[12] Therefore, the aim of the study was to assess ventricular function among controlled hypertensive patients with and without periodontitis.

Material and methods

The present study protocol was submitted to and approved by the Central Ethics Committee at the NITTE Deemed to be University, Mangalore, India (NU/CEC/2018/0197), and registered in the Clinical Trials Registry – India (CTRI) (CTRI/2018/08/015383). The study was conducted following the ethical regulations of the 1975 Declaration of Helsinki, as revised in 2013, and reported following the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology). The study was conducted between August 2017 and August 2019.

The sample size (N) was calculated based on the following formula (Equation 1):

$$N = Z^2 \times (1-\alpha) \;/\; 2 \times PQ \;/\; d^2 \qquad (1)$$

where:
N – sample size;
$Z^2 \times (1-\alpha) / 2$ – confidence interval;
P – estimated proportion;
Q = 1 – P; and
d – desired precision.

The confidence interval was 95%, the estimated proportion was 0.091 and the desired precision was 5%.

The current cross-sectional study involved 2 groups with an equal number of participants, 75 each. Group A consisted of controlled hypertensive patients without periodontitis. Patients with the sulcus depth of less than or equal to 3 mm, no bleeding on probing (BOP), no clinical attachment loss (CAL), no visual inflammation, and no mobility were considered as non-periodontitis patients. Group B involved controlled hypertensive patients with periodontitis. Patients with periodontitis had more than 30% of the sites with CAL ≥ 5 mm and probing pocket depth (PPD) ≥ 4 mm, as measured with the Hu-Friedy UNC-15 Color-Coded probe (Hu-Friedy, Chicago, USA) (stage III periodontitis).[13]

Stage I controlled hypertensive patients with normal LVF according to the Joint National Committee (JNC 8) hypertension guidelines,[14] with a minimum of 20 natural teeth and a body mass index (BMI) of 18.5–24.9 kg/m^2 were included in the study. Patients with any other congenital and developmental cardiac illness, malignancies and tumors, and self-reported current and former tobacco and alcohol users were excluded from the study (Fig. 1).

Informed verbal and written consent was obtained from the selected patients prior to the study. One examiner (S.S.) was involved in the periodontal examination, and another examiner (S.K.) was involved in performing 2D echocardiography.

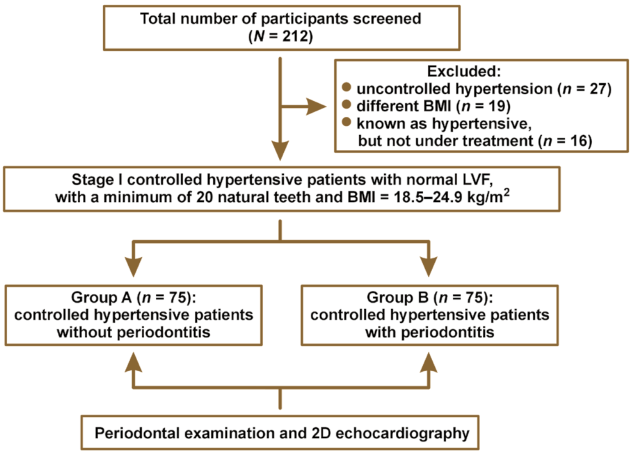

Fig. 1. Flowchart – study methodology

BMI – body mass index; LVF – left ventricular function; 2D echocardiography – two-dimensional echocardiography.

Calculation of the PISA score

The complete periodontal examination consisted in assessing PPD and CAL, calculated on all 6 surfaces of natural teeth, BOP, and recession, which was then used to calculate the PISA score.[15] A freely downloadable spreadsheet to calculate the PISA score was obtained from www.parsprototo.info.

Calculation of GLS

The patients were allowed to relax for 30 min before starting the procedure. A Doppler echocardiogram (EPIQ 7C 2.0.2.; Philips Healthcare, Andover, USA) with a transducer probe (X5-1 3D probe; frequency: 93 Hz) was used to evaluate the myocardial strain. The transducer probe was placed by a blinded examiner on the chest or abdomen of the subject to get various views of the heart using ultrasound. The GLS imaging is made in standard apical two-, three- and four-chamber views. The images were displayed on a monitor for real-time viewing and recorded by marking 3 specific points (the apex, the base and the septum, i.e., LVAP4, LVAP2 and LVAP3) manually within the myocardium. LVAP4, LVAP2, and LVAP3 represent LV apical four-chamber, two-chamber, and three-chamber views, respectively, and tracking these Doppler points enables the measurement of the strain rate by the machine (Fig. 2). LVAP4 represents the longitudinal strain in a four-chamber view, LVAP2 represents the longitudinal strain in a two-chamber view and LVAP3 rep-

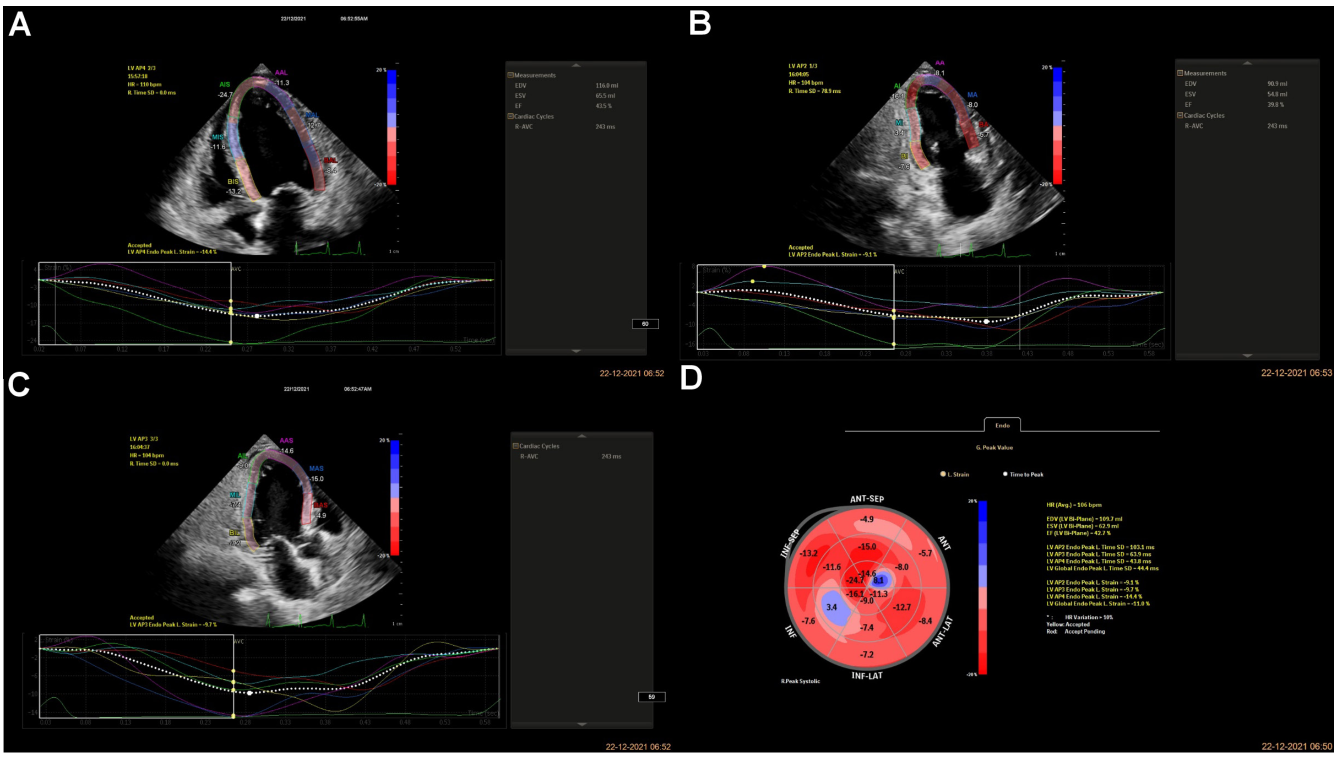

Fig. 2. A – left ventricular apical four-chamber view (LVAP4); B – left ventricular apical two-chamber view (LVAP2); C – left ventricular apical three-chamber view (LVAP3); D – bull's eye view

resents the longitudinal strain in a three-chamber view. The GLS is calculated as the average from all segments to measure the global LVF. The GLS rate was derived using the following formula (Equation 2) and expressed as percentage[16]:

$$\varepsilon = \frac{\Delta\varepsilon}{\Delta t} = \frac{\left(\frac{\Delta L}{L0}\right)}{\Delta t} = \frac{\left(\frac{\Delta L}{\Delta t}\right)}{L0} = \frac{\Delta V}{L0} \qquad (2)$$

where:
ε – strain;
t – time instance;
L – instantaneous length;
L0 – baseline length; and
ΔV = velocity gradient in the segment;
$\Delta\varepsilon = \varepsilon \times \Delta t$; $\Delta t = \Delta\varepsilon / \varepsilon$; $\Delta L = L - L0$.

Statistical analysis

The collected data was entered into a Microsoft Excel spreadsheet and analyzed using IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA). Descriptive data was presented as number and percentage (*n* (%)) for categorical variables, and as mean and standard deviation (*M* ±*SD*) and median and interquartile range (*Me* (*IQR*)) for continuous variables. The Mann–Whitney *U* test was used to compare the variables between the study groups. Spearman's correlation test was used to assess the correlation between GLS and the study variables. Multiple linear regression was used to develop a model to predict GLS based on the study variables. A *p*-value <0.05was considered statistically significant.

Results

The mean age of the patients in group A was 52.80 ±5.25 years, and in group B, 53.00 ±5.70 years. The comparison of the variables between the study groups is presented in Table 1. Group B had significantly higher mean PPD, CAL, BOP, and PISA scores as compared to group A ($p < 0.001$).

Multiple linear regression modeling suggested that LVAP4 was a statistically significant predictor of GLS in

Table 1. Comparison of the variables between the study groups

Variable	Group	*M* ±*SD*	Min	Max	*Me* (*IQR*)	Mann –Whitney *U* test	
						U	*p*-value
Age [years]	A	52.80 ±5.25	43.00	60.00	53.00 (48.00–57.00)	2,778.0	0.900
	B	53.00 ±5.70	45.00	60.00	52.00 (48.00–59.00)		
BMI [kg/m²]	A	22.82 ±1.79	18.60	24.90	23.50 (21.60–24.30)	2,591.5	0.410
	B	22.55 ±1.94	18.50	24.90	23.50 (21.40–24.10)		
Systolic blood pressure [mm Hg]	A	131.36 ±6.70	116.00	140.00	132.00 (126.00–136.00)	2,734.5	0.770
	B	131.28 ±7.75	112.00	140.00	132.00 (126.00–138.00)		
Diastolic blood pressure [mm Hg]	A	75.04 ±4.55	68.00	86.00	74.00 (70.00–78.00)	2,336.5	0.070
	B	76.61 ±5.22	70.00	90.00	78.00 (72.00–80.00)		
PISA [mm²]	A	74.57 ±12.04	41.10	98.70	76.60 (65.40–83.20)	0.0	<0.001*
	B	411.46 ±74.37	175.50	541.30	426.40 (364.50–462.90)		
GLS [%]	A	−16.55 ±4.18	−22.30	−15.40	−16.80 (−17.70 to −15.70)	2,491.0	0.230
	B	−17.43 ±1.98	−23.40	−14.40	−17.40 (−18.60 to −15.80)		
HR [bpm]	A	75.27 ±6.60	60.00	88.00	77.00 (69.00–80.00)	2,797.0	0.950
	B	75.04 ±7.40	54.00	88.00	76.00 (69.00–80.00)		

M – mean; *SD* – standard deviation; min – minimum; max – maximum; *Me* – median; *IQR* – interquartile range; PISA – periodontal inflamed surface area; GLS – global longitudinal strain; HR – heart rate; * statistically significant.

group A, and LVAP3, LVAP4 and EF were statistically significant predictors of GLS in group B (Tables 2 and 3). In both groups, age showed a significant positive correlation with GLS, whereas PISA had no association with GLS ($p > 0.05$) (Tables 4 and 5). The correlations are presented in Fig. 3 and 4. The regression model resulted in 88% ($R = 0.88$) of change in GLS for group B (Table 2).

Discussion

A recent systematic review and meta-analysis, which included 7 studies and 4,307 participants, revealed a significant relationship between periodontitis and peripheral artery disease.[17] However, very few studies have studied the relationship between the inflammatory component of periodontal disease and cardiovascular diseases. The PISA score reflects the surface area of the bleeding pocket epithelium, thereby quantifying the probability of a systemic inflammatory burden due to periodontitis, which may influence the development of atherosclerosis, LV dysfunction, or other cardiovascular complications.[18] Among hypertensive patients, there is a significant decrease in the GLS rate and the global circumferential deformation due to subclinical LV dysfunction with the preserved EF.[15] A possible mechanism could be the sustained elevated blood pressure in the arteries, which may affect LV relaxation and result in gradual LV hypertrophy, thereby leading to early diastolic dysfunction through the impaired LV longitudinal strain.[19] Cytokines and nitro-oxidative stress have a direct negative inotropic effect, and thereby can promote myocardial ischemia, apoptosis and LV dysfunction.[20] Thus, inflammatory-driven oxidative stress leads to vascular and myocardial dysfunction and fibrosis. Similarly, non-coronary inflammation, such as in chronic obstructive pulmonary disease, Kawasaki disease, rheumatoid arthritis, and others, has also been shown to influence LV dysfunction through the modestly altered inflammatory markers, leading to changes in ventricular mechanics. However, the mechanism through which periodontitis affects LVF has not been explained. It could be attributed to gram-negative anaerobic bacteria that invade superficial and deeper gingival tissues, thereby

Table 2. Multiple linear regression to predict the global longitudinal strain (GLS) in groups A and B

Group	Variable	Unstandardized coefficients		Standardized coefficients	*t*	*p*-value	95% *CI*	
		B	*SE*	B			lower bound	upper bound
A	constant	1.090	7.823	–	0.139	0.890	−14.521	16.700
	PISA	−0.069	0.036	−0.204	−1.950	0.055	−0.140	0.002
	LVAP2	0.503	0.300	0.227	1.677	0.098	−0.096	1.103
	LVAP3	0.026	0.076	0.036	0.341	0.734	−0.127	0.179
	LVAP4	0.721	0.263	0.344	2.744	0.008*	0.197	1.246
	EF	0.003	0.087	0.004	0.036	0.971	−0.170	0.176
	HR	0.114	0.064	0.185	1.801	0.076	−0.012	0.241
B	constant	1.601	1.512	–	1.059	0.293	−1.416	4.618
	PISA	7.541E-5	0.001	0.003	0.067	0.947	−0.002	0.002
	LVAP2	0.022	0.013	0.072	1.723	0.089	−0.003	0.047
	LVAP3	0.411	0.060	0.408	6.876	0.001*	0.292	0.530
	LVAP4	0.457	0.045	0.558	10.204	0.001*	0.368	0.547
	EF	−0.068	0.017	−0.185	−3.968	0.001*	−0.102	−0.034
	HR	0.009	0.012	0.035	0.759	0.451	0.015	0.034

SE – standard error; *CI* – confidence interval; LVAP2 – left ventricular apical two-chamber view; LVAP3 – left ventricular apical three-chamber view; LVAP4 – left ventricular apical four-chamber view; EF – ejection fraction; * statistically significant.
Dependent variable: GLS. Group A: $F (5, 69) = 6.05$; $p < 0.001$; $R^2 = 0.31$. Group B: $F (4, 70) = 131.96$; $p < 0.001$; $R^2 = 0.88$.

Table 3. Comparison of the left ventricular (LV) ejection fraction (EF) between the study groups

Group	n	*M* ±*SD*	Mean difference	95% *CI* of the difference		*t*	df	*p*-value
				lower bound	upper bound			
A	75	67.60 ±5.48	0.40	−1.36	2.16	0.450	148	0.660
B	75	67.20 ±5.42						

df – degrees of freedom. Independent samples *t* test.

Table 4. Correlations between the study variables in group A

Variable	Statistical parameter	PISA	LVAP2	LVAP3	LVAP4	GLS	HR	EF
Age	correlation coefficient	0.18	0.72	0.62	0.70	0.75	0.04	−0.60
	p-value	0.120	<0.001*	<0.001*	<0.001*	<0.001*	0.710	<0.001*
BMI	correlation coefficient	0.07	0.04	0.18	0.08	0.19	−0.03	0.01
	p-value	0.580	0.730	0.120	0.480	0.100	0.780	0.950
Systolic blood pressure	correlation coefficient	0.20	−0.15	−0.07	−0.32	−0.17	−0.18	−0.03
	p-value	0.090	0.200	0.580	0.005*	0.140	0.120	0.820
Diastolic blood pressure	correlation coefficient	−0.12	−0.01	0.12	0.09	0.06	−0.15	−0.10
	p-value	0.310	0.930	0.310	0.420	0.580	0.200	0.390
Mean PPD	correlation coefficient	0.35	0.04	−0.01	−0.01	0.05	−0.12	0.05
	p-value	0.002*	0.730	0.950	0.950	0.690	0.310	0.690
Mean CAL	correlation coefficient	0.35	0.27	0.26	0.10	0.24	0.03	−0.05
	p-value	0.002*	0.020*	0.030*	0.400	0.040*	0.830	0.700
Mean BOP	correlation coefficient	0.36	0.12	0.06	−0.11	0.02	0.02	−0.13
	p-value	0.002*	0.300	0.590	0.330	0.850	0.870	0.290
PISA	correlation coefficient	1.00	0.22	0.06	0.06	−0.12	0.08	−0.11
	p-value	–	0.064	0.630	0.580	0.313	0.520	0.340
LVAP2	correlation coefficient	–	1.00	0.26	0.56	0.38	−0.002	−0.46
	p-value	–	–	0.023*	<0.001*	0.001*	0.980	<0.001*
LVAP3	correlation coefficient	–	–	1.00	0.20	0.15	−0.04	−0.80
	p-value	–	–	–	0.070	0.200	0.710	0.490
LVAP4	correlation coefficient	–	–	–	1.00	0.45	−0.07	−0.36
	p-value	–	–	–	–	<0.001*	0.520	<0.001*
GLS	correlation coefficient	–	–	–	–	1.00	0.14	−0.61
	p-value	–	–	–	–	–	0.220	<0.001*
HR	correlation coefficient	–	–	–	–	–	1.00	−0.03
	p-value	–	–	–	–	–	–	0.790

PPD – probing pocket depth; CAL – clinical attachment loss; BOP – bleeding on probing; * statistically significant (Spearman's correlation test).

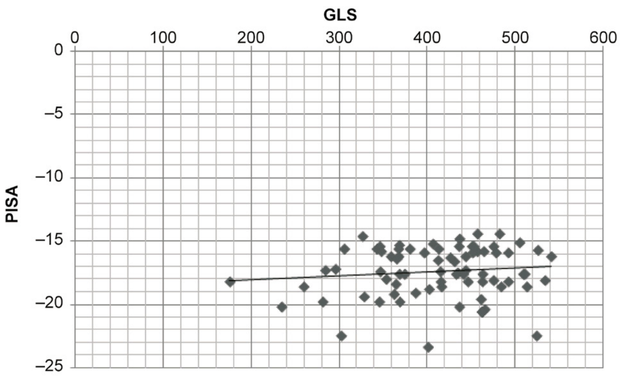

Fig. 3. Scatter plot – correlation between periodontal inflamed surface area (PISA) and global longitudinal strain (GLS)

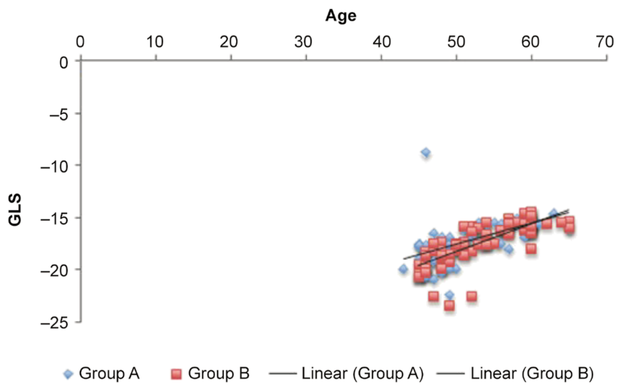

Fig. 4. Scatter plot – correlation between age and global longitudinal strain (GLS)

making it possible for the products of the pathogens to be released into the systemic circulation, which causes bacteremia.[21] Akamatsu et al. noted that there were specific, elevated concentrations of interleukin-17A (IL-17A) and interferon-gamma (IFN-γ) mRNA expression in the hearts of mice after the injection of *Porphyromonas gingivalis*, and that the systemic inflammatory burden could induce myocarditis.[22]

This study utilized 2D echocardiography to obtain the GLS value, the most sensitive echocardiographic marker which decreases monotonically with progressive cardiac dysfunction. It is the GLS that typically varies according to age, gender and LV loading conditions.[23] Defining abnormal GLS is not straightforward. However, based on a meta-analysis study, the average clinical range of GLS varies considerably from −15.9 to −22.1 (the percentage shortening

Table 5. Correlations between the study variables in group B

Variable	Statistical parameter	PISA	LVAP2	LVAP3	LVAP4	GLS	HR	EF
Age	correlation coefficient	−0.02	0.62	0.77	0.65	0.83	−0.09	−0.45
	p-value	0.850	<0.001*	<0.001*	<0.001*	<0.001*	0.430	<0.001*
BMI	correlation coefficient	0.18	0.19	0.21	0.11	0.20	0.05	0.06
	p-value	0.120	0.110	0.070	0.340	0.090	0.670	0.600
Systolic blood pressure	correlation coefficient	−0.09	0.29	0.23	0.31	0.29	−0.07	−0.17
	p-value	0.420	0.010*	0.040*	0.008*	0.010*	0.530	0.140
Diastolic blood pressure	correlation coefficient	−0.02	0.12	0.04	−0.09	0.003	−0.03	−0.04
	p-value	0.870	0.310	0.770	0.460	0.980	0.800	0.730
Mean PPD	correlation coefficient	0.43	0.04	0.07	0.19	0.07	−0.08	0.04
	p-value	<0.001*	0.750	0.570	0.100	0.530	0.510	0.710
Mean CAL	correlation coefficient	0.09	0.18	0.06	0.09	0.18	0.07	−0.31
	p-value	0.470	0.120	0.610	0.470	0.120	0.580	0.007*
Mean BOP	correlation coefficient	0.49	0.17	0.14	0.07	0.10	−0.02	−0.17
	p-value	<0.001*	0.140	0.230	0.550	0.410	0.870	0.140
PISA	correlation coefficient	–	0.02	0.16	0.33	0.11	0.02	−0.13
	p-value	–	0.860	0.150	0.780	0.310	0.850	0.240
LVAP2	correlation coefficient	––	–	0.09	0.14	0.19	0.12	−0.20
	p-value	–	–	0.430	0.200	0.090	0.910	0.860
LVAP3	correlation coefficient	–	–	–	0.56	0.80	0.17	−0.38
	p-value	–	–	–	<0.001*	<0.001*	0.120	<0.001*
LVAP4	correlation coefficient	–	–	–	–	0.82	−0.18	−0.17
	p-value	–	–	–	–	<0.001*	0.100	0.130
GLS	correlation coefficient	–	–	–	–	–	−0.02	−0.47
	p-value	–	–	–	–	–	0.850	<0.001*
HR	correlation coefficient	–	–	–	–	–	–	0.13
	p-value	–	–	–	–	–	–	0.240

* statistically significant (Spearman's correlation test).

of the long axis dimension), whereas a score of more than −15.9 is considered to be clinically abnormal strain.[24]

In this study, differences in the GLS scores between the A and B groups were not statistically significant ($p > 0.05$) (Table 1). However, when clinically abnormal strain values are considered, i.e., GLS scores > −15.9, group B patients were experiencing an abnormal strain of −14.40 and the value was greater as compared to group A patients (Table 1), which is likely an indication of mild LV strain for the increased PISA scores. As previously reported by Park et al., a 1% abnormal strain on the myocardium increases the patient's mortality risk by 5%.[25] Thus, in the current study, group B patients could be at risk of developing a future cardiovascular event. The PISA score is a better indicator of the inflammatory load experienced by patients than PPD and CAL, as the latter refer only to linear measurements and do not indicate the systemic effects of the inflammation. Moreover, as periodontitis is of microbial origin, PISA could be considered an indicator of microbial infection.[26] Also, the generalized linear model analysis revealed a significant association between PISA and the level of high-sensitivity C-reactive protein (hs-CRP); therefore, PISA could be an effective index for estimating the effect of periodontitis on the whole body, enabling medical-dental cooperation.[27] This could explain why the inflammation caused by periodontitis in group B patients resulted in a systemic inflammatory burden, and hence led to mild LV dysfunction.

Based on the depicted values, the mean PISA score was significantly higher in group B (411.46 ±74.37) as compared to Group A (74.57 ±12.04) ($p < 0.05$) (Table 1). Furthermore, the mean PISA scores did not show any statistically significant influence on the GLS scores ($p > 0.05$) (Tables 4 and 5). This might be due to the fact that PISA did not reach a threshold level that could influence myocardial activity. Besides, the recruited patients were on anti-hypertensive therapy, which would have improved myocardial activity, thereby limiting the influence of PISA on the GLS scores.[15,28] However, this can be further verified using longitudinal studies. Also, multivariate linear regression analysis showed that for group B, 87.4% of the variation in the GLS rate was predicted due to the independent variable (PISA), whereby for every one-unit increase in the PISA score, there was an abnormal strain rate alteration of 7.54×10^{-5} (Table 2). Hence, in our study, it is pertinent that an increase in the GLS score, which is suggestive of the abnormal strain pattern, can result in an increased mortality risk in the future.

The current study also strengthened the following existing evidence:

1) Despite the preserved EF, subclinical LV dysfunction was observed, which proves that the GLS assessment is more sensitive to early LV dysfunction in comparison with the EF assessment.[29]

2) Age could be considered an independent risk factor for LV dysfunction, since aging results in decreased cardiac deformation, and ultimately in increased LV stiffness.[30]

The current research could be improved by including the lipid profiles of the individuals and the systemic inflammatory component driven by periodontal disease, as well as by optimizing the use of hypertensive medication. Additionally, it is advised to use a larger sample size.

Conclusions

The current study attempted to explore the influence of periodontitis on ventricular mechanics by utilizing 2D echocardiography. Although the observed values were not statistically indicative of any relationship between the 2 variables, clinically undetectable inflammatory insult due to periodontitis might have resulted in mildly altered ventricular mechanics. However, future longitudinal studies with long-term follow-ups could prove a stronger association between periodontitis and LVF.

Trial registration

The research was registered in the Clinical Trials Registry – India (CTRI) (CTRI/2018/08/015383).

Ethics approval and consent to participate

The present study protocol was approved by the Central Ethics Committee at the NITTE Deemed to be University, Mangalore, India (NU/CEC/2018/0197). Informed verbal and written consent was obtained from the selected patients prior to 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.

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Original paper

Evaluation of the wound-healing potential of the kiwifruit extract by assessing its effects on human gingival fibroblasts and angiogenesis

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

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Abstract

Background. Oral surgery and surgical interventions in the field of periodontology require the uneventful wound healing of soft tissues to be successful. However, since the oral cavity is exposed to saliva and is a niche for oral microorganisms, wound healing can be delayed, causing pain and discomfort to the patient. Hence, a great deal of interest has shifted to the study of agents that can enhance wound-healing processes.

Objectives. The aim of the present study was to evaluate the oral wound-healing potential of the *Actinidia deliciosa* (kiwifruit) extract by assessing its effects on the viability, proliferation and migration of human gingival fibroblasts, and its ability to enhance angiogenesis in the chicken chorioallantoic membrane (CAM).

Material and methods. The collection of the *A. deliciosa* extract and experimentation were carried out in the Maratha Mandal Dental College, Belgavi, India. Human gingival fibroblasts were cultured in a suitable medium and treated with 6.25 µg, 12.5 µg or 25 µg of the ethanolic kiwifruit extract. The MTT cell viability and scratch assays were then carried out in vitro. To determine the angiogenic potential of the extract, solutions of concentrations of 10 µg/mL, 50 µg/mL and 100 µg/mL were transferred to sterile Whatman™ filter paper disks, which were placed on the CAM of 5-day-old Leghorn chicken eggs.

Results. The ethanolic extract of *A. deliciosa* was not cytotoxic to gingival fibroblasts at a dosage of 6.25 µg/mL. In addition, the scratch assay demonstrated the migration of the fibroblasts and the closure of an artificially created wound within 24 h, which is similar to in vivo wound closure. The CAM assay showed that the kiwifruit extract concentrations of 10 µg/mL and 50 µg/mL showed a moderate pro-angiogenic effect, and a concentration of 100 µg/mL exerted a strong pro-angiogenic effect.

Conclusions. The results demonstrate that the kiwifruit extract is safe to use, can enhance the proliferation and migration of human gingival fibroblasts, and promotes angiogenesis, making it a suitable oral wound-healing agent.

Keywords: wound healing, plant extracts, herbal therapy, angiogenesis stimulators, gingiva

Introduction

Wound healing is a complex process that aims to repair and reconstruct the lost or injured tissues.[1] In the oral mucosa, wound healing differs from the healing of the skin, as it proceeds faster and rarely scars.[2] Studies have found that the fibroblasts isolated from the oral mucosa and skin have several inherent differences, including their capability to remodel tissues, secretion patterns of certain extracellular matrix (ECM) molecules, cellular migration, cellular adhesion, response to growth factors, and differential expression of ECM receptors.[3,4]

Oral surgery and surgical interventions in periodontology include flap surgeries, tooth extractions, implant placement, and the treatment of conditions such as oral abscesses and ulcers. These treatment modalities require the uneventful wound healing of soft tissues to be successful. However, since the oral cavity is exposed to saliva and is a niche for oral microorganisms, wound healing may be delayed, causing pain and discomfort to the patient. Therefore, the main objective of holistic oral wound management is to facilitate healing in the shortest time possible, with minimal pain and discomfort.[5] Hence, a great deal of interest has shifted to studying agents that can enhance wound healing.

Therapeutic wound-healing agents can be either natural or synthetic; they are characterized by different levels of efficacy and clinical acceptance. Various agents that promote wound healing and tissue regeneration have come into focus in recent times, such as bone morphogenic proteins (BMPs),[6] lactoferrin,[7] platelet-rich fibrin,[8] vitamin D,[9] melatonin, and taurine, among many others. These agents have shown excellent results when implemented after oral and implant surgeries.[6–9] Due to their clinical efficacy, simplicity and affordability, the healing agents derived from plants and herbs have assumed a central role in wound care. Hence, various herbal products have been evaluated as local therapeutic agents for the enhancement of wound healing.[10]

The kiwifruit belongs to the genus *Actinidia* (*Actinidiaceae*). It is rich in vitamin C, carotenoids, tannins, and saponins, among many other active compounds. The nutrient and biologically-active phytochemical content of the fruit has stimulated investigations into its antioxidant and anti-inflammatory potential in preventing cardiovascular disease, cancer, and other degenerative disorders.[11–13] Furthermore, the *Actinidia deliciosa* extract has been found to be effective against gram-positive and gram-negative bacteria.[14,15]

Studies have shown that the kiwifruit extract accelerates scar detachment and helps in the healing of burn wounds. These effects may be attributed to the presence of some compounds in the kiwifruit, such as ascorbic acid, which acts as a scavenger, and the strong protein-degrading enzyme actinidin, which makes it a good anti-escharotic agent, as well as carotenoids, which have strong antioxidant properties.[12–14]

The objective of the present study was to evaluate the efficacy of the kiwifruit extract in wound healing by assessing the migration and proliferation of human gingival fibroblasts, and evaluating angiogenesis, with the aim of using this natural agent to improve oral wound healing.

Material and methods

The study design was approved by the Institutional Review Board (IRB) at the Maratha Mandal Dental College, Belgavi, India, before commencement (No. BCD Exam/509/2019-20), and it was performed in accordance with the Declaration of Helsinki ethical standards.

Procurement and preparation of the extract

Pure *A. deliciosa* extract was obtained in its powder form from an International Organization of Standardization (ISO) certified natural extract manufacturer (Navchetana Kendra Health Care Private Limited, New Delhi, India). An ethanolic extract of the dried kiwifruit powder was prepared using the maceration technique. A total of 5 g of the extract powder was added to 50 mL of 70% ethanol in a 250-milliliter sterile conical flask, which was plugged with sterile cotton and placed in a shaking incubator (Köttermann, Uetze, Germany) at 200 rpm for 24 h. The filtration process was carried out using a muslin cloth, and it was repeated 3 times to obtain a clear ethanolic solution of the extract.

Human gingival fibroblast cell culture

Human gingival fibroblasts (Adult (HGF) ATCC® PCS-201-018™) were procured (Department of Microbiology, the Maratha Mandal Dental College, Belgavi, India). They were initially centrifuged at 225 g for 5 min and washed in phosphate-buffered saline (PBS). The cells were maintained in Eagle's Minimal Essential Medium (EMEM) supplemented with 10% fetal calf serum (FCS), antibiotics (5% of a mixture of gentamicin (10 μg), 100 U/mL penicillin and 100 g/mL streptomycin) and L-glutamine (200 mM), and were incubated at 37°C in a humidified atmosphere of 5% carbon dioxide (CO_2) and 95% oxygen (O_2) for 48 h. The medium was replaced every few hours until confluence was reached.[16]

MTT cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay is a non-radioactive colorimetric system that measures cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity.

A commercially available cell proliferation kit was purchased (Sigma-Aldrich, Bangalore, India), from which a stock solution was prepared by adding 5 mg of MTT to 1 mL of PBS. The test compound was assessed by the spectrophotometric determination of the conversion of MTT into the formazan blue product by viable cells. Three quantities of the kiwifruit extract – 6.25 µg, 12.5 µg and 25 µg – were diluted in 1 mL of dimethyl sulfoxide (DMSO), and then added to wells containing 100 µL of the previously prepared MTT stock solution. For the control, only DMSO was added to the stock solution, without the addition of the kiwifruit extract. The human gingival fibroblasts were then added to the test solutions and incubated for 4 h. After incubation, the supernatant was carefully aspirated. The precipitated crystals of formazan blue were dissolved by adding 100 µL of DMSO. The optical density (OD) was measured at a wavelength of 570 nm, using an LISA Plus microplate reader (Aspen Medical Group, Inc., Deakin, Australia).[17]

The mean of 6 readings was used to calculate the results by using the following formula (Equation 1):

$$\text{surviving cells [\%]} = \frac{\text{mean OD of the test compound}}{\text{mean OD of the control}} \times 100 \quad (1)$$

where:
OD – optical density.

Scratch assay

The scratch assay was performed to study cell migration and the cell–cell interaction. This was achieved by seeding human gingival fibroblasts onto a 24-well cell culture plate and creating a linear scratch in the confluent cell monolayer with a 200-microliter pipette tip. Cell debris was washed out with plain EMEM. The ethanolic extract of *A. deliciosa* at concentrations of 6.25 µg/mL, 12.5 µg/mL and 25 µg/mL was used for the experimental groups, and 0.5% DMSO was used for the control group. Following the addition of the extract, images of the cellular gap were captured after 10 h, 12 h, 16 h, and 24 h. The study was completed when the scratch wound was fully closed.[18]

CAM assay

The chicken chorioallantoic membrane (CAM) bioassay was used to evaluate the potential toxicity and pro-angiogenic influence of the ethanolic kiwifruit extract at the vascular level of CAM.[15] Fertilized white Leghorn chicken eggs were purchased (Tamil Egg Hatchery, Chennai, India) and incubated in a Multiquip Incubator (E2) (Multiquip Inc., Cypress, USA) at 37°C and 60% humidity for 24 h. The egg tray was automatically tilted at a 450-degree angle every 30 min to mimic natural processes. A small window was made in the shell on the 5th day of the chick embryo development under aseptic conditions, and sterile Whatman™ filter paper disks containing 10 µL of PBS and the ethanolic kiwifruit extract at different concentrations were carefully placed onto the CAM of the eggs. The test compound was prepared at concentrations of 10 µg/mL, 50 µg/mL and 100 µg/mL in 0.2% DMSO. A DMSO control was carried out simultaneously for the comparison with the angiogenic activity of the ethanolic extract. The window was re-sealed with adhesive tape, and the eggs were incubated for 48 h. The windows were reopened after 48 h of incubation, which was the 8th day of the chick embryo development, and close-up photographs were taken to capture the images of CAM. The images were analyzed for the changes in the angiogenesis process induced by the test compound. All experiments were conducted in duplicate.[19]

Results

MTT cell viability assay

The kiwifruit extract dosage of 25 µg/mL resulted in the lowest viability (76%) and the highest cell death (24%) of the human gingival fibroblasts. Meanwhile, a dosage of 12.5 µg/mL resulted in 93% cell viability and 7% cell death, and treatment with the 6.25 µg/mL dosage led to 100% cell viability and 0% cell death (Fig. 1 and 2, Table 1).

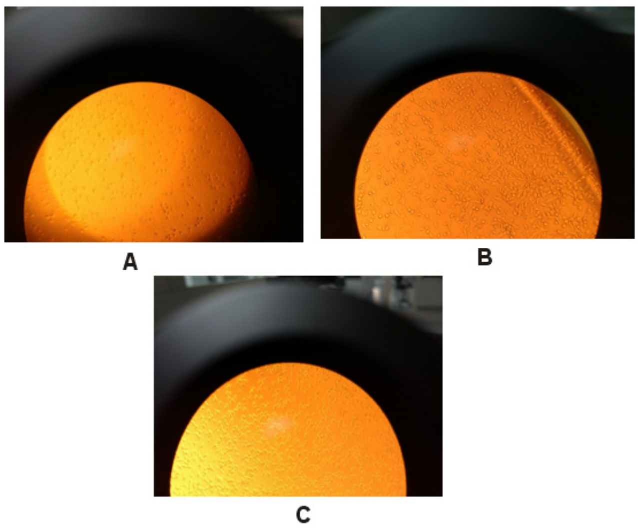

Fig. 1. A – 76% of human gingival fibroblasts viable after treatment with 25 µg/mL of the ethanolic kiwifruit extract; B – 93% viable cells after treatment with 12.5 µg/mL; C – 100% viable cells after treatment with 6.5 µg/mL, as observed under light microscope

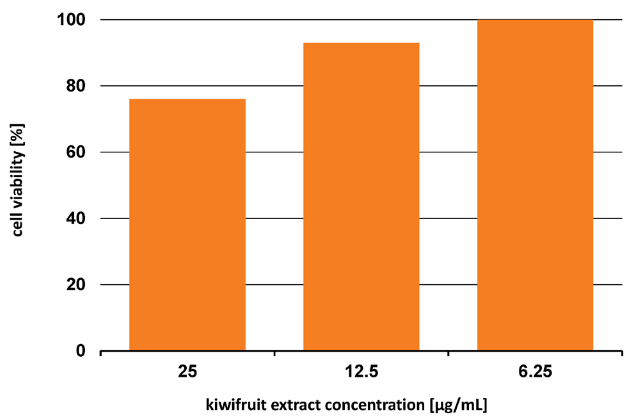

Fig. 2. Cell viability at different concentrations of the kiwifruit extract

Table 1. Concentrations of the kiwifruit extract and the percentages of viable and dead cells

Extract concentration [µg/mL]	OD values	Mean OD value	Cell viability [%]	Cell death [%]
25	0.548 0.552 0.542 0.550 0.556 0.549	0.549	76	24
12.5	0.674 0.669 0.662 0.671 0.672 0.668	0.669	93	17
6.5	0.751 0.742 0.754 0.741 0.755 0.749	0.748	100	0

OD – optical density.

Scratch assay

Adult human gingival fibroblasts were used in the scratch assay. The experimental results showed that 6.25 µg/mL of the extract caused significant cellular mobilization. Indeed, microscopic analysis after 10 h revealed migration distances from the top of the scratch of 235.45 µm and 286.86 µm, and migration distances of 208.86 µm and 290.47 µm from the bottom of the scratch (Fig. 3). After 16 h, cell migration increased rapidly, with a nearly full closure of the scratch. A complete closure of the scratch was achieved after 24 h. The time taken to close the gap was plotted against the percentage of cell migration that occurred (Fig. 4).

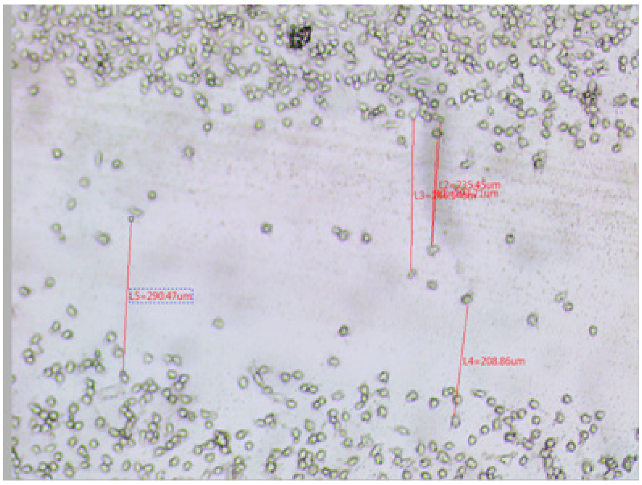

Fig. 3. Human fibroblast cells migrating over the scratch at 10 h after treatment with the kiwifruit extract

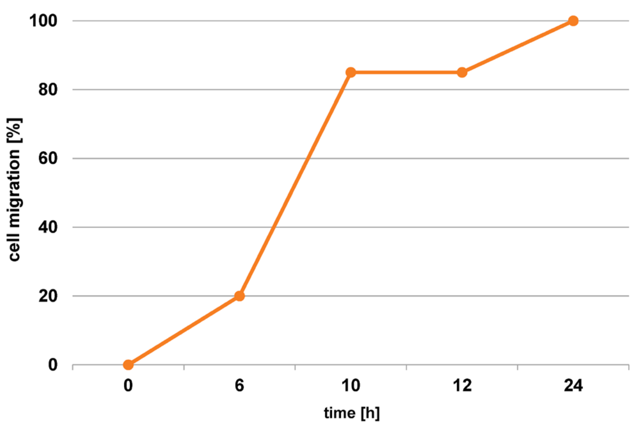

Fig. 4. Time taken for wound closure

CAM assay

The microscopic examination revealed that blood vessel formation was stimulated in all the eggs loaded with the ethanolic kiwi extract. The stimulation was concentration-dependent, with moderate stimulation achieved at 10 µg/mL and 50 µg/mL, and strong stimulation achieved at 100 µg/mL (Fig. 5, Table 2).

Table 2. Concentrations of the kiwifruit extract and the effect on blood vessel proliferation

Extract concentration [µg/mL]	Replicate	Budding of blood vessels
100	R1	+++
	R2	+++
50	R3	++
	R4	++
10	R5	++
	R6	++
Negative control	R7	alive, no change

+++ blood vessels strongly formed; ++ blood vessels moderately formed.

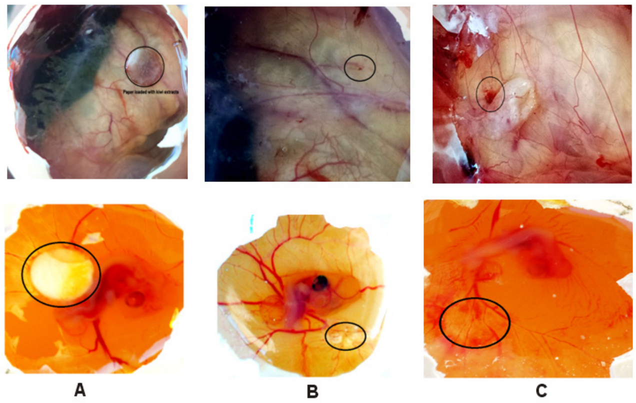

Fig. 5. A – sterile paper loaded with the kiwifruit extract on the chorioallantoic membrane (CAM); B – moderate formation of blood capillaries observed at 10 µg/mL; C – strong formation of blood capillaries observed at 100 µg/mL (camera and microscopic images)

Discussion

There has been a recent surge of the evidence supporting the health protective properties of plant-derived compounds, which has resulted in great interest and justifiable scientific assessment.[20] Various plant extracts are known to contain carotenoids, polyphenols, vitamin A, and ascorbic acid (vitamin C); they are easy to obtain and economical, and have negligible side effects on humans and the environment.[10,20] As such, natural compounds could enhance wound healing following oral surgery and surgical interventions in periodontology. Therefore, the present study evaluated the effects of the kiwifruit extract on the proliferation of human gingival fibroblasts, as well as its impact on angiogenesis in CAM.

Actinidia deliciosa is known to contain antibacterial agents, scavenging agents and proteolytic enzymes, which have been shown to improve wound healing.[17,21,22] Furthermore, the *A. deliciosa* extract acts against a wide spectrum of microorganisms, including *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Salmonella typhi*.[17,23] Moreover, the qualitative phytochemical analysis of the kiwifruit extract revealed the presence of carotenoids, alkaloids, flavonoids, saponins, tannins, and terpenoids, which shows its high antioxidant capacity.[23]

Phytochemicals such as vitamin A, vitamin C and carotenoids are required for the synthesis of collagen, a fibrous protein that helps to reinforce connective tissues, which hold the structures of the body together. They are also highly effective in protecting cells against damage by free radicals. It has been observed that after gingival wounding, the amount of myofibroblasts, a fibroblast subtype that is mainly involved in collagen synthesis and fibrillar remodeling, increases.[2,3] While myofibroblasts are not usually found in healthy, mature connective tissues, their formation is enhanced and modulated by wound-healing factors, such as cytokines, enzymes and vitamins.[4] Ascorbic acid, at lower concentrations, increases chondrogenesis and osteogenesis, and enhances the secretion of growth factors, anti-inflammatory cytokines and the factors related to bone metabolism. Therefore, ascorbic acid is indispensable in wound healing. Since the kiwifruit extract is a rich source of vitamin C and carotenoids, it was imperative to analyze its effects on wound healing, and assess the viability and functionality of human gingival fibroblasts by using specialized tests, such as the MTT and scratch assays.

Angiogenesis is an important phase of the wound-healing process, and greatly influences the remodeling stage of tissue regeneration and repair.[3–5] For this reason, the effect of the kiwifruit extract on neovascularization were evaluated using the CAM assay.

This study is the first of its kind to assess the oral healing potential of the kiwifruit extract. Previous studies established its role in the management of cutaneous and burn wounds, but to the best of our knowledge, no study has evaluated the effects of the kiwifruit extract on human gingival fibroblasts with the MTT, scratch and CAM assays.

The results of this study revealed that 6.25 µg/mL of the kiwifruit extract caused no gingival fibroblast cellular destruction, while treatment with 12.5 µg/mL resulted in 93% cell viability and 7% cell death, and 25 µg/mL of the extract led to 76% cell viability and 24% cell death. Therefore, the results demonstrated that the ethanolic kiwifruit extract concentrations ranging from 6.25 µg/mL to 12.5 µg/mL exerted minimal toxic effects on human gingival fibroblasts, whereas 25 µg/mL was toxic to human gingival fibroblasts.

Previous studies showed that the kiwifruit extract had a broad anti-inflammatory effect on human monocytes, and could stimulate cellular proliferation in normal human keratinocytes (NHK) and immortalized human keratinocytes (HaCaT).[24,25] The kiwifruit is well-known for containing compounds such as β-carotene and ascorbic acid, both of which are toxic to fibroblasts at high concentrations (>10 mg/L) and reduce oxidative stress in skin fibroblasts at low concentrations (1 mg/mL).[26] This may help to explain the results obtained in the cell viability test, which showed the toxic effect of the kiwifruit extract on human gingival fibroblasts at a higher concentration (25 µg/mL).

The scratch assay demonstrated the migration of fibroblasts and the closure of the artificially created, 606.51-micrometer-wide scratch within 24 h, which is similar to in vivo wound closure. Indeed, cell migration rapidly increased 10 h post-treatment and fibroblasts completely closed the wound at 24 h. The initiation of fibroblast proliferation depends on specific proteins from ECM, including elastin, laminin and collagen, and proteoglycans, such as hyaluronan.[27]

Studies have demonstrated that ascorbic acid promotes the synthesis of ECM proteins, especially collagen, at lower concentrations, but is toxic to fibroblasts at higher concentrations.[26,28] This correlates with the results of the current study, indicating that the ascorbic acid present in the kiwifruit extract increased the proliferation of human gingival fibroblasts, thereby promoting oral wound healing.

Regarding angiogenesis, the CAM assay showed no negative impact on capillary formation when using minute concentrations of the extract. At concentrations of 10 μg/mL, 50 μg/mL and 100 μg/mL, the extract stimulated abundant blood vessel formation, which progressively decreased with a decreasing concentration, demonstrating a strong pro-angiogenic effect. Low concentrations of plant-derived compounds, specifically β-carotene, β-cryptoxanthin, lutein, and zeaxanthin, are known to stimulate the production of vascular endothelial growth factor (VEGF),[10,25,29] which improves angiogenesis during wound healing by propagating the growth and migration of endothelial cells through ECM. These compounds are found in abundance in the kiwifruit extract, which may explain the results of the CAM assay.[11,12,25]

It is safe to conclude that a concentration range of 6.25–12.5 μg/mL of the ethanolic kiwifruit extract accelerates fibroblast migration with minimum cytotoxicity and with good pro-angiogenic action, thus indicating its use as an oral wound-healing agent.

In the present study, only the ethanolic kiwifruit extract was used for the MTT cell viability, scratch and CAM assays. The use of different solvents or other kiwifruit extracts may result in different outcomes, as demonstrated in a study on the *Piper longum* extracts, which showed that different solvents (aqueous, alcohol, acetone) resulted in variable outcomes in the CAM assay.[30] Therefore, there is scope for further studies using different solvents to verify and compare the concentration ranges achieved in the present study. The results from this study could be applied in the development of mucosal patches, ointments, gels, and solutions, to enhance wound healing following oral and gingival surgeries.

Conclusions

To conclude, the present study showed that the kiwifruit extract enhanced the proliferation and migration of human gingival fibroblasts, and promoted angiogenesis in vitro. The enhanced wound healing activity may be due to the individual activity or synergistic effects of the bioactive molecules present in the kiwifruit extract. This data opens up the possibility of using the kiwifruit extract to effectively manage oral wounds, especially those caused by oral surgery and periodontal procedures, to prevent patient morbidity and discomfort. However, there were several limitations to this study. The study was conducted in vitro, and further in vivo investigations are required, as is the use of different kiwifruit extract concentrations and solvents. Such studies will confirm the suitability of the kiwifruit extract as an effective oral wound-healing agent, and are required before it can be used for this purpose.

Ethics approval and consent to participate

The study design was approved by the Institutional Review Board (IRB) at the Maratha Mandal Dental College, Belgavi, India, before commencement (No. BCD Exam/509/2019-20), and it was performed in accordance with the Declaration of Helsinki ethical standards.

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

Impact of glutaraldehyde cross-linking on the advanced platelet-rich fibrin membrane: Macroscopic, conventional light microscopic and scanning electron microscopic analysis

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

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Abstract

Background. Advanced platelet-rich fibrin (A-PRF) is a biopolymer that releases growth factors to facilitate healing. Along with other barrier membranes, the A-PRF membrane has proven to be beneficial in guided tissue regeneration (GTR) treatment. The cross-linking of the A-PRF membrane with glutaraldehyde (GLUT) has been attempted previously, and has been shown to prolong its degradation time and improve its mechanical properties. In the present study, the effects of GLUT cross-linking on macroscopic changes in the A-PRF membrane were assessed, and microscopic features were analyzed using a light microscope and a scanning electron microscope (SEM).

Objectives. The aim of the present study was to evaluate and compare the effects of GLUT cross-linking on the A-PRF membrane through the macroscopic, microscopic and SEM examinations.

Material and methods. A total of 18 human A-PRF membrane samples were prepared, half of which were treated with 0.1% GLUT, and the remaining were left untreated. The macroscopic measurements of the samples included weight, length and thickness, while specimen slides were prepared for light microscopic evaluation and SEM analysis.

Results. The GLUT cross-linked membranes weighed more and were thicker than the non-cross-linked membranes, but there was no change in length. Light microscopic images showed fewer cells at the head and tail, though cells were abundant in the body of the A-PRF membrane. The images acquired using SEM showed fibrin strands of greater thickness, but fewer interspersed cell bodies in the cross-linked membranes.

Conclusions. This in vitro study revealed an increase in thickness and cross-linking fiber density along with the presence of viable cells in the GLUT-treated A-PRF membrane, which may prove its effectiveness in healing or serving as a barrier membrane in clinical trials.

Keywords: glutaraldehyde, advanced platelet-rich fibrin, cross-linking

Introduction

Advanced platelet-rich fibrin (A-PRF) is a second-generation, self-clotting, platelet-concentrated, blood-derived biomaterial rich in white blood cells. The material is prepared through the contact activation of intrinsic coagulation pathways, using low-speed centrifugation, without the need for additional coagulation factors.[1] It provides the sustained release of high concentrations of growth factors, which enhance collagen synthesis and supplement progenitor cells.[2] Studies have demonstrated the use of PRF membranes in the treatment of periodontal osseous defects with open-flap debridement[3] to cover the defects filled with bone grafts, potentially also functioning as a guided tissue regeneration (GTR) membrane.[4] Furthermore, PRF has been used as an adjunct to GTR membranes to facilitate vascularization, to guide the migration of epithelial cells, and to provide growth factors to enhance soft and hard tissue regeneration.[5] A retrospective clinical trial revealed promising bone gain associated with the guided bone regeneration (GBR) procedure, which included combining membranes, bone grafts and PRF for vertical and horizontal bone augmentation.[6] However, the major shortcomings of PRF include its rapid degradability and poor mechanical strength. Thus, the use of PRF membranes on their own has not proven to be popular in GTR treatment, as they cannot maintain space for tissue regeneration or prevent the migration of epithelial cells for a sufficient period of time.[5] Fortunately, biopolymers can be modified to achieve an ideal combination of mechanical properties, geometry and surface chemistry by subjecting them to cross-linking treatment.[7] Therefore, overcoming the shortcomings of PRF membranes would allow clinicians to use them successfully in GBR and GTR therapies.

The 2 aldehyde groups of glutaraldehyde (GLUT) can react with free amino groups of polypeptide chains to form Schiff bases, which leads to the cross-linking of biological tissue materials.[8] A 0.1% cross-linked amniotic membrane had the maximum cross-linking of its amino groups, resulting in a significant increase in thermal stability and an increased degradation time.[9] Furthermore, no morphological abnormalities and good cell viability were observed at low concentrations.[9] In addition, GLUT vapor treatment was used to maintain the bioactivity of the electrospun fibrinogen scaffolds.[10]

The toxicity of GLUT is related to its concentration, the duration of cross-linking and its release from the cross-linked material. The exposure of cell cultures to 0.1% GLUT cross-linked amniotic membranes for 24 h led to a significant decrease in the number of viable corneal epithelial cells as compared to the groups exposed to lower concentrations.[9] Gelatin films cross-linked with a 0.1% GLUT solution for 24 h released 2 wt% after 1 week, which increased up to 9 wt% after 4 weeks.[11] In our previous study, A-PRF membranes cross-linked with 0.1% GLUT for 10 min showed an increased mechanical strength and a prolonged degradation time, and a 0.083 wt% GLUT release after 24 h, with no further release over the following 2 weeks.[12]

Previous results have opened up the possibility of using GLUT cross-linked A-PRF as an efficient GTR membrane. However, its ultrastructure requires extensive evaluation before it can be used as a clinical application in periodontal regeneration. Therefore, this study aimed to evaluate and compare the macroscopic parameters, light microscope-acquired images and scanning electron microscope (SEM)-acquired images of GLUT cross-linked A-PRF membranes and non-cross-linked A-PRF membranes.

Material and methods

Nine systemically healthy male volunteers, aged 20–35 years, were selected from the outpatient department of the Bapuji Dental College and Hospital, Davangere, India. Volunteers who smoked tobacco products or who were receiving anticoagulant therapy were excluded. The study design and consent forms for all the procedures performed on human subjects were approved by the board of the institutional Ethical Committee (No. BDC/Exam/509/2019-20). The purpose of the study was verbally explained to the volunteers, and written consent to participate in the study was obtained before its commencement. Blood was drawn from each volunteer (20 mL) to prepare 2 A-PRF membranes.

Preparation of advanced platelet-rich fibrin clots

The A-PRF samples were prepared according to the protocol developed by Ghanaati et al.[13] Venous blood (20 mL) was collected via the cubital vein, using Vacutainer™ tubes (Becton Dickinson Company, Tokyo Japan), and transferred to A-PRF tubes (Zhejiang Gongdong Medical Technology Co., Ltd., Taizhou, China). The A-PRF tubes were immediately centrifuged at 1,500 rpm for 14 min, using the A-PRF12 system (Dragon Laboratory Instruments Ltd., Beijing, China). Centrifugation led to the formation of 3 separate layers, including a red blood cell (RBC) base, an acellular plasma supernatant (platelet-poor plasma – PPF) and a PRF clot in the middle. The clot was separated from the A-PRF tube and the membrane was prepared by compression in an A-PRF expression box, which maintained a uniform size and a standard thickness of 1 mm for each clot. The prepared membranes were divided into experimental and control samples, and immediately subjected to the required treatment for macroscopic and microscopic evaluation to avoid the loss of functional capacity and cell integration due to a prolonged bench time.[14]

Glutaraldehyde cross-linking of platelet-rich fibrin

A total of 18 A-PRF membranes were prepared, out of which 9 membranes were cross-linked with 10 mL of a 0.1% GLUT solution in phosphate-buffered saline (PBS) at a pH of 7.4 for 10 min at room temperature. The remaining 9 non-cross-linked membranes were used as control samples.[12] Macroscopic evaluation, and light microscopy and SEM imaging were performed in triplicate to avoid bias.

Macroscopic analysis

The length and weight of A-PRF membranes were measured using a ruler and a digital balance (Contech Instruments Limited, Mumbai, India), respectively, as shown in Fig. 1A and 1B. Both the experimental and control samples were placed on a pre-weighed sterile microscope slide for weight and length measurements.

Light microscopic analysis

The ultrastructure of fibrin and the distribution of cell bodies within the A-PRF membranes were examined using the Olympus CX21i light microscope (Olympus, New Delhi, India). Before analysis, the A-PRF membranes were fixed in 10% neutral buffered formalin for 24 h at room temperature for paraffin inclusion.[15] The membranes were stored on a microscope slide during fixation to avoid distortion. Successive sections (4-micrometer-thich) were collected along the center of the long axis of the membranes and stained with hematoxylin and eosin (H&E). Each section was divided into 3 areas of equal size, including head/face (proximal), body (center) and tail (distal), as shown in Fig 1C. The center of each area was observed to analyze the distribution of visible cell bodies (marked in dark purple) under ×100, ×200 and ×400 magnification. For semi-quantitative evaluation, manual cell body counting was performed at ×200 magnification by an oral pathologist. The total number of cell bodies counted was used to compare the distribution among the 3 areas of the membrane.[16] Most of the cells were concentrated in the body area.

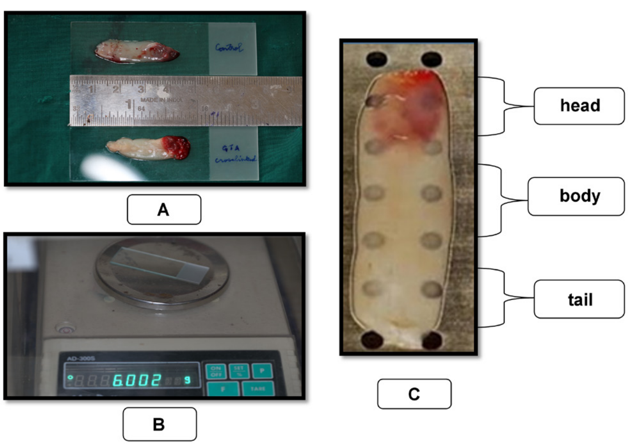

Fig. 1. A, B – evaluation of macroscopic parameters; C – light microscopy procedure

Scanning electron microscopic analysis

The surface microstructure, topography, morphology, and compositional information of the A-PRF membranes were examined using the FE-SEM SU6600 (Hitachi High Technologies America, Inc., Hillsboro, USA). Before analysis, The samples were dehydrated by passing each specimen through a series of graded ethanol-water mixtures, and then dried by the critical point method. After drying, the samples were sputter-coated with gold and examined under SEM.[17] Images were acquired using magnifications ranging from 2.5 kX to 10 kX.

Statistical analysis

The macroscopic and microscopic (semi-quantitative) evaluation values were tabulated and subjected to statistical analysis. Comparisons were made between the cross-linked and control samples using Student's independent *t* test. Values of $p < 0.001$, $p < 0.05$ and $p > 0.05$ were considered highly significant, significant and non-significant, respectively.

Results

Macroscopic analysis

A significant weight increase was recorded in the GLUT cross-linked samples as compared to the non-cross-linked controls ($p = 0.010$). Meanwhile, the lengths of both the experimental and control samples were almost identical, and were not found to be significantly different ($p = 0.100$) (Table 1).

Table 1. Comparison of macroscopic parameters between the test and control groups

Parameter	Group	*n*	*M* ±*SD*	*SEM*	95% *CI*	*p*-value
Weight [g]	test	3	0.32 ±0.15	0.008	0.01–0.07	0.010*
	control	3	0.28 ±0.10	0.005		
Length [cm]	test	3	3.43 ±0.05	0.033	0.03–0.23	0.100
	control	3	3.33 ±0.05	0.033		

Test group: glutaraldehyde (GLUT) cross-linked advanced platelet-rich fibrin (A-PRF) membranes. Control group: Non-cross-linked A-PRF membranes. *M* – mean; *SD* – standard deviation; *SEM* – standard error of the mean; *CI* – confidence interval; * statistically significant ($p < 0.05$).

Light microscopic analysis

Due to the H&E stain, the nuclei of the cell bodies stained a dark purple. Erythrocytes and the cytoplasm of the cell bodies were reddish pink or dark pink. Meanwhile, the fibrin network appeared light pink.

Head/face

The clusters of erythrocytes and residual cell bodies could be observed entrapped within the fibrin network in both the experimental and control samples. In addition, the fibrin network was denser in the experimental samples, and there were significantly fewer cell bodies in the experimental samples than in the control ones ($p = 0.002$) (Fig. 2, Table 2).

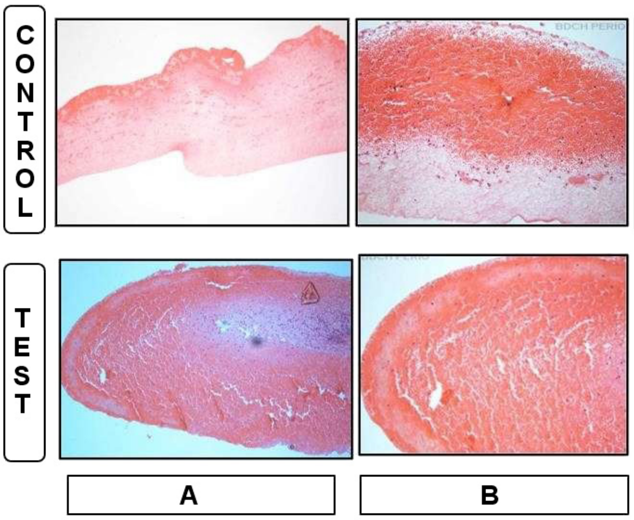

Fig. 2. Microscopic pictures of the head/face of the advanced platelet-rich fibrin (A-PRF) membrane samples of the control and test groups

A – ×100 magnification; B – ×200 magnification.

Table 2. Comparison of cellular body distribution between the test and control groups

Parameter	Group	*n*	*M* ±*SD*	*SEM*	95% *CI*	*p*-value
Head	test	3	148.30 ±7.63	4.40	−60.6 to −26.0	0.002*
	control	3	191.60 ±7.63	4.40		
Body	test	3	316.60 ±76.30	44.09	−597.5 to −335.7	0.001**
	control	3	783.30 ±28.80	16.66		
Tail	test	3	58.30 ±7.63	4.40	−40.6 to −6.0	0.020*
	control	3	81.60 ±7.63	4.40		

* statistically significant ($p < 0.05$); ** highly statistically significant ($p < 0.001$).

Body

The vast majority of cell bodies (95%) were present in the body of the A-PRF membranes, and a mature fibrin network, as well as cellular components, were well separated from each other in both the experimental and control samples. Most of the cell bodies observed were lymphocytes and plasma cells, particularly those entrapped within the fibrin network. The clusters of erythrocytes were interspersed between cell bodies in the control group. In the experimental group, cellular components were separated by fibrillar bands. Furthermore, the fibrin network was abundant between cells, and there was a significantly lower cell density in the experimental samples as compared to the control samples ($p = 0.001$). Cells appeared intact and had a normal shape in both the experimental and control samples (Fig. 3, Table 2).

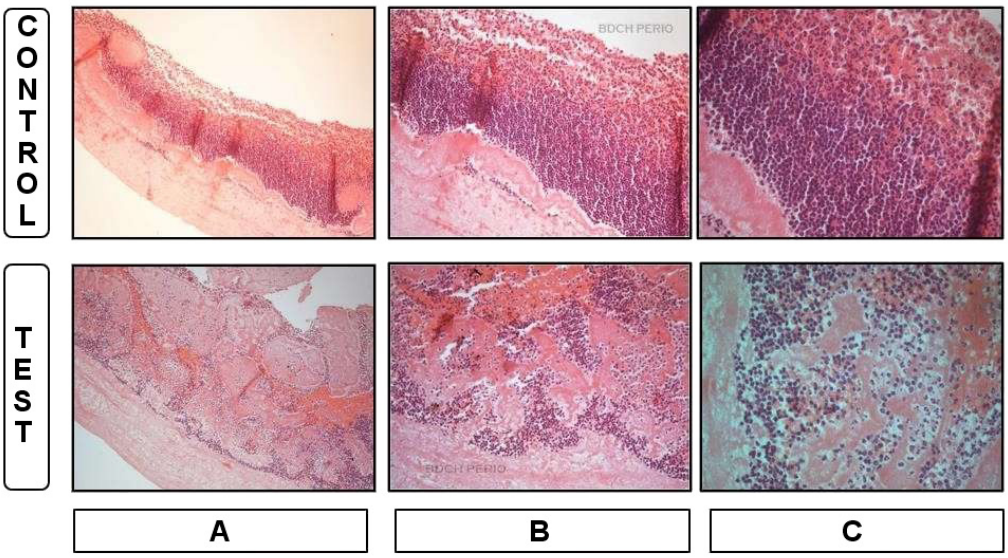

Fig. 3. Microscopic pictures of the body of the advanced platelet-rich fibrin (A-PRF) membrane samples of the control and test groups

A – ×100 magnification; B – ×200 magnification; C – ×400 magnification.

Tail

The tail of the membrane was bordered by a homogenous well-organized fibrin mesh structure with continuous integrity, which enclosed the clusters of erythrocytes in both the experimental and control samples. In addition, residual cell bodies were entrapped within the fibrin network and between the clusters of erythrocytes. Loosely arranged fibers and a textured fibrin network were visible in the experimental samples, while the control group had a fibrin network of densely organized parallel strands. The number of cells was significantly higher in the control group than in the experimental group (p = 0.020) (Fig. 4, Table 2).

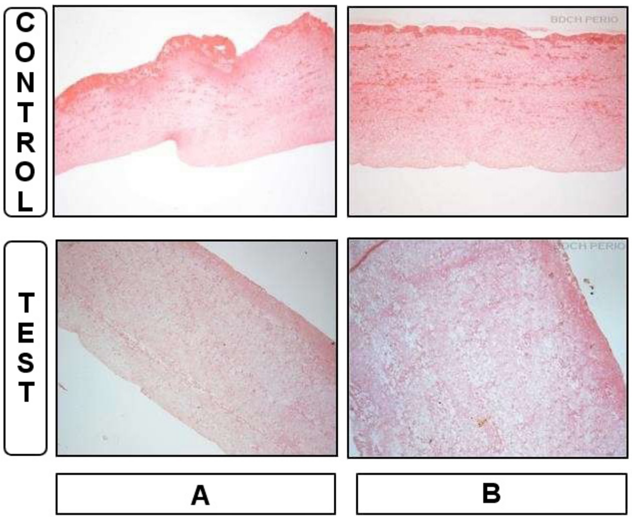

Fig. 4. Microscopic pictures of the tail of the advanced platelet-rich fibrin (A-PRF) membrane samples of the control and test groups
A – ×100 magnification; B – ×200 magnification.

Scanning electron microscopic analysis

The SEM analysis provided information on the surface topography, fibrin network, cell content, and internal structure of each membrane within a small area of great depth. At a low magnification of 2.5 kX, the control samples had a highly irregular surface with overlying spherical structures, which were identified as leukocytes. A dense aggregate of platelets resting on a mature fibrin background was also observed. Platelet morphology appeared to be modified by the aggregation and clotting process. The experimental samples had a dense fibrin matrix with surface morphology similar to that of medical gauze and had fewer cellular components as compared to the control samples.

At a higher magnification of 10 kX, a continuous dense fibrin network with entrapped cellular structures was observed in the control samples. Meanwhile, a thick fibrin fiber network of a porous structure was observed in the experimental samples, where intact leukocytes and platelets were found to be entrapped (Fig. 5).

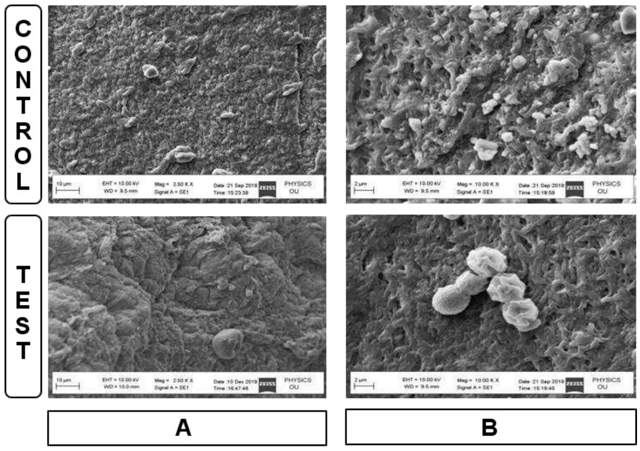

Fig. 5. Scanning electron microscopy (SEM) pictures of the advanced platelet-rich fibrin (A-PRF) membrane samples of the control and test groups
A – 2.5 kX magnification; B – 10 kX magnification.

Discussion

The concentration of RBC tends to decrease in elderly patients, which makes it easier to separate the plasma layers and a larger modified A-PRF clot can be produced as compared to younger patients. The PRF membranes produced from female patients tend to be 17% larger than those produced from male patients due to reduced RBC concentration and hematocrit levels in peripheral blood.[18] Although the platelet concentration is higher in females than males, females show cyclical variations. Indeed, the platelet concentration appears to align with the estrogen levels and reduces before the onset of menstruation, before peaking at the follicular and luteal phases.[19] Low platelet and leukocyte counts were observed in tobacco smokers due to alterations in various hematological parameters.[20] Meanwhile, female smokers reach menopause early, which leads to reduced platelet activation and a higher mean platelet count due to falling estrogen levels.[21] In diabetic patients, osmotic effects, an increase in the glucose levels and the presence of glucose metabolites in blood lead to higher levels of larger and more reactive platelets.[22] In addition, patients undergoing anticoagulant therapy experience a delay in coagulation cascade activity, which adversely affects fibrin clot formation.[23] To avoid bias, all the volunteers included in this study were males, young, non-smokers, systemically healthy, and not receiving anticoagulant therapy.

Modifications to the PRF preparation procedure by adopting the low-speed centrifugation concept (LSCC), a process that employs low G-forces, led to the development of A-PRF. In comparison with standard PRF (S-PRF), the LSCC process results in enhanced neutrophilic granulocytes, which contribute to the differentiation of monocytes into macrophages, an increase in the cytokine levels, higher growth factor concentrations, and an increased release bone morphogenetic proteins. Such improvement leads to earlier vascularization, and more rapid soft and hard tissue regeneration.[13] Injectable PRF (i-PRF) is also prepared using LSCC, and has higher growth factor levels and increased anti-inflammatory efficacy; however, it is a liquid and flowable form of PRF, which is used either alone or in combination with other biomaterials.[24]

In its membranous form, A-PRF can be placed on surgical wounds for healing purposes, as it acts as a fibrin glue due to its adhesive mechanical properties and biological functions.[2] It has been shown in vitro that fibrin membranes may be better scaffolds for the proliferation of periosteal and osseous cells than collagen membranes.[25] Therefore, a previously published study examined the mechanical properties and degradation time of GLUT cross-linked membranes with the intention of using them as a more viable and cost-effective barrier membrane and scaffold for periodontal regeneration.[12]

Glutaraldehyde is one of the most commonly used cross-linking agents. Perpetuated exposure to a high concentration of GLUT leads to incomplete cross-linking due to rapid initial polymerization at the surface of the fibers. This prevents GLUT from accessing molecules within the deeper areas of the fibrin structure for the initiation of cross-linking by the creation of nucleation sites or a static hindrance, which leads to the consumption of large amounts of free GLUT and subsequent high toxicity.[26] Amniotic membrane matrices treated with low-concentration GLUT displayed good compatibility with human corneal epithelial cells.[9] As such, the A-PRF membranes obtained in this study were treated with 0.1% GLUT for 10 min to achieve membrane cross-linking without toxic effects. Indeed, only minimal GLUT was released after 24 h, and no further release was observed over the following 2 weeks.[12]

In this study, an increase in the weight of the GLUT cross-linked samples was observed, which could be attributed to the penetration of GLUT into the membrane. Glutaraldehyde primarily fixes the surface of the fibrin fibers to create a polymeric network and initiate cross-linking. Therefore, GLUT cross-linking increases the molecular weight of proteins and alters their conformation.[26] No change was observed in the membrane length between the experimental and control groups following GLUT cross-linking. However, a shrinkage of A-PRF membranes was observed when they were subjected to heat compression at 90°C and 120°C for 15 s and 2 s, respectively.[27]

Due to higher centrifugal force, platelets and inflammatory cells within the fibrin scaffold were observed mainly in the proximal portion. The LSCC used to produce A-PRF membranes adopts low G-forces to reduce cell pull-down, which increases the number of leukocytes trapped within the fibrin matrix of the top layer.[13] Also, the top layer at the center of the membrane may have been stabilized when the clot was pressurized in the PRF box, which may explain the denser distribution of cells in the body of the experimental and control membranes. The reduced centrifugal force used for A-PRF preparation caused lower leukocyte infiltration into the RBC fraction, meaning more were present in the adjacent fibrin clot, although the RBC fraction was not eliminated.[13] As a result, more leukocytes were found at the center, and erythrocyte clusters were observed in the head and tail of the A-PRF membrane under the light microscope. However, this is in contrast to other studies, which showed a greater concentration of cell bodies in the head of the membrane and homogenous platelet aggregate distribution in the tail of the membrane.[15,16] Such differences may be due to variations in the equipment and materials used, such as the centrifuge and tubes, and variations in the protocols, such as the tube temperature, the rpm rate, gravitational force, or the duration of centrifugation.[16]

The fibrin network of the GLUT-treated A-PRF membranes was slightly thick and loose, and had a reticular arrangement, which was in contrast to the control samples. The presence of such thick fibrin fibers and high serum retention within its spaces could enhance the volume-to-surface area ratio, and may give GLUT cross-linked membranes an advantage over normal A-PRF membranes in relation to their degradation rate.[12] In the tails of the experimental and control membranes, fibrin strands were condensed and adhered to each other, which may have been due to the compression of the fibrin matrix during membrane preparation.[15]

The thick, dense and regular pattern found in the fibrin network of the experimental samples is supported by the results of a previous study, where increased tensile strength and toughness were observed in GLUT cross-linked A-PRF membranes.[12] The SEM analysis also revealed greater porosity in the GLUT-treated membranes, which might facilitate cell migration and the release of growth factors, both of which are important attributes of a biological healing matrix.[17] Furthermore, the SEM analysis of the experimental membranes supports the light microscopy findings regarding a decrease in cellular components and an increase in fibrin density. In the non-cross-linked A-PRF membranes, leukocytes and platelets were well distributed and were not clumped. In addition, the control matrices had lower porosity, which is consistent with the findings of Isobe et al., who found A-PRF membranes to contain denser and more mature fibrin threads.[28]

The SEM-acquired images revealed the presence of mutilated cells in the control membranes at a lower magnification. These damaged cells raised concerns, as they may release pro-inflammatory mediators. In this regard, the ratio of activated and preserved cells to damaged cells may play an important role in regulating the inflammatory processes clinically, which needs to be addressed in future work.[16] Acceleration rates of more than 1 m/s^2 cause damage to cells and alter their distribution. The A-PRF centrifuge used in this study has a rate of 3 m/s^2,[16] which may explain the appearance of mutilated/aggregated platelets in the control membranes at lower magnification.

In the experimental group, the thick and dense fibrin matrix may have disguised these modified cells due to the focus being at a greater depth and the limited surface area coverage at a higher magnification.

The microscopic (light microscope/SEM) evaluations of A-PRF membranes are scarce,[15,17] and most studies have used conventional microscopy, which requires the dehydration or drying of specimens. Such preparation processes may cause topographical, morphological and compositional changes to the cells and fibers of A-PRF membranes. Therefore, future research using environmental SEM,[29] or with modifications to conventional SEM by using the wet cover method[30] will allow the observation of wet specimens and may overcome the limitations of conventional SEM.

Conclusions

The present descriptive in vitro study throws light on the possibility of using GLUT-treated A-PRF as a GTR membrane. It reaffirms the impact of GLUT as a cross-linking agent in the production of a resilient and viable biological membrane. However, the research is still at a primitive stage. Further evaluation of the cytotoxic effects and compatibility with viable regenerative cells is required before its clinical application in the field of periodontal regeneration.

Ethics approval and consent to participate

The study design and consent forms for all the procedures performed on human subjects were approved by the board of the institutional Ethical Committee at the Bapuji Dental College and Hospital, Davangere, India (No. BDC/Exam/509/2019-20). The purpose of the study was verbally explained to the volunteers, and written consent to participate in the study was obtained before its commencement. The study was performed in accordance with the Declaration of Helsinki ethical standards.

Data availability

All data generated and/or analyzed during this study is included in this published article.

Consent for publication

Not applicable.

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Original paper

Evaluation of the remineralization and antibacterial effect of natural versus synthetic materials on deep carious dentin: A randomized controlled trial

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Abstract

Background. A variety of natural and synthetic agents have recently been used in clinical trials to arrest dentin caries.

Objectives. The aim of the present study was to explore the remineralization and antibacterial effect of natural (propolis, hesperidin) vs. synthetic (silver diamine fluoride (SDF)) agents on deep carious dentin.

Material and methods. A total of 64 human molar teeth with Class I caries were randomly distributed into 4 groups: control group; propolis group; hesperidin group; and SDF group. The cavities were prepared using the stepwise caries removal technique, and then covered with the materials to be tested. The samples were taken from the carious lesions before and after treatment to evaluate the antibacterial effect. Then, the teeth were restored with a glass ionomer cement (GIC). Digital X-rays were taken to assess remineralization and the antibacterial effect after 6 and 12 weeks.

Results. The highest value of radiodensity was observed in the propolis group (46.44 ±9.65 HU), while the lowest value was noted in the hesperidin group (12.62 ±5.86 HU). The bacterial count in the propolis group was 1,280.00 ±1,480.54 CFU/mL at baseline, which was not significantly higher than the value measured after 6 weeks (574.00 ±642.48 CFU/mL; $p = 0.153$), whereas in the hesperidin group, the mean value of the bacterial count at baseline (3,166.67 ±1,940.79) was not much higher as compared to the value obtained at 6 weeks (2,983.33 ±1,705.77) ($p = 0.150$).

Conclusions. In comparison with SDF, propolis and hesperidin agents showed promising effects in terms of remineralization of carious dental tissue and hindering the progression of caries.

Keywords: propolis, remineralization, dental caries, hesperidin, silver diamine fluoride, antibacterial effect

Introduction

In an era aiming to conserve dental tooth tissue, strategies for the management of dental lesions, both carious and non-carious, have evolved to preserve tooth structure and minimize removal. In the oral cavity, teeth are often affected by various alterations, such as dental caries, abnormal teeth, enamel hypoplasia, supernumerary teeth, and dental agenesis. Dental caries is characterized by areas of regional tooth tissue deterioration caused by bacterial flora and acid products which cause demineralization of tooth structure and proliferation of bacterial colonies. On the other hand, non-carious lesions are characterized by a loss of hard dental tissue due to nonbacterial factors, especially near the cement-enamel-junction. These lesions frequently cause dentin hypersensitivity and can promote the development of additional caries. While the major difference between carious and non-carious lesions is the bacterial factor, a non-carious lesion may result in retentive niches in which bacteria can flourish and create a pathological biofilm, which in turn promotes further carious lesions.[1] Risk factors for the development of caries include all conditions that can cause difficulties in oral hygiene and also orthodontic brackets. Other conditions that can cause oral hygiene problems include Psychiatric disorders, genetic disorders or genetics syndromes and other debilitating conditions connected with manual labor.[2,3] Temporomandibular disorders and other mouth pain conditions such as lesions of oral mucosa may limit mouth opening and hinder proper hygiene.

Once a lesion develops, conservative modalities should be employed to treat or heal the lesion.[4–6] Among the conservative modalities used include the treatment of hypersensitivity using diode laser treatment. Conservative modalities for caries management also include recognition of risk factors, as well as early detection and remineralization of incipient carious lesions.[7,8] Carious lesions can progress over time and develop into the pulp and as they penetrate deeper into tooth structure, thus preservation of the remaining dentin becomes of paramount importance. In addition, the neutralization of the remaining bacterial virulence is essential to arrest the lesion and prevent further pathogenesis. When the caries is destructive, extraction of the affected tooth is essential. This can change occlusal forces and increase the difficulty of teeth brushing, thus it is fundamental to rehabilitate all teeth as soon as possible. Rehabilitation can includes the use of an implant or a removable prosthesis.[9,10]

Bacterial presence in deep cavities is reduced following incomplete carious dentin removal and tooth sealing when compared to traditional complete carious dentin removal. Additionally, the dentin beneath the interim restoration develops features of an inactive caries lesion (dry, hard, and dark).[11] This has led to the evolution of selective caries removal modalities.

One of the main techniques used for selective caries removal is stepwise excavation. This technique involves retaining a layer of carious soft dentin above the pulp. After that, a protective liner is placed, and the tooth is sealed for a specific duration (30–45 days). The goal of this technique is to stimulate the development of tertiary dentin prior to complete excavation, hence decreasing the likelihood of pulp exposure.[12,13] Concern for the remaining bacteria in deep carious lesions remains controversial. While some authors have emphasized the seal as the primary factor for success of this technique, others have recommended the use of antibacterial agents for management of the remaining viable bacteria. The use of natural agents, such as tannins, terpenoids, flavonoids, alkaloids, have become very popular for the prevention of caries. The antibacterial activities of these agents have been found to be useful against dental caries.[14,15] Propolis, a bee product, has drawn interest for its safety and biological activity due to its polyphenolic compounds. Propolis is a flavonoid derivative resinous obtained from honeybees and from sprouts, exudates of trees and other parts of plants. It has antimicrobial, antitumor, anesthetic, anti-inflammatory, antiviral, and healing properties. Polyphenols are another promising natural agents due to their ability to interfere with several pathways involved in the pathogenesis of some inflammatory conditions such as TMJ-related inflammation.[16] The antibacterial activity of some natural materials (aloe vera and propolis) after minimally invasive hand excavation of dental caries have also been investigated. An antibacterial effect was assessed by visual assessment of the total number of viable bacterial unit-forming colonies. In a clinical trial comparing the effect of aloe vera and propolis on bacteria in hand excavated lesions, a significant amount of bacteria were left behind after hand excavation, but cavities treated with aloe vera and propolis extracts had a significant reduction in bacterial counts when compared to the control group.[17] Another comparison was conducted between the effects of propolis fluoride (PPF) and nano-silver fluoride (NSF) to silver diamine fluoride (SDF) varnish for inhibiting *Streptococcus mutans* and *Enterococcus faecalis* biofilm formation. The results of this analysis confirmed that NSF and PPF fluoride-based varnishes had greater antibacterial effects than SDF fluoride-based varnish.[18]

Hesperidin is a flavonoid extracted from citrus fruits. Hesperidin has anti-inflammatory, anti-microbial, antioxidant, and collagen cross-linking effects which limit the development of caries and enhance the remineralization process.[19] Hesperidin has been added to dental adhesive in three different ratios producing four experimental adhesive groups (control, 0.2%, 0.5%, and 1%). Results showed that 0.5 wt% HPN incorporated dental adhesives could achieve a promising antibacterial effect without adversely affecting the adhesive characteristics.[20]

A variety of synthetic agents have also been used in clinical trials to arrest dentin caries. Some antimicrobial agents contain silver (Ag) such as silver diamine fluoride

(SDF), which has a bactericidal effect and is affordable, effective, safe, and easy to use for stopping the progression of carries.[21] Clinical trials have found that a topically applied SDF solution inhibits demineralization. Additionally, SDF inhibits the growth of cariogenic bacteria in biofilms, effectively prevents the development of caries, and meets the standards of the WHO Millennium Goals and the US Institute.[22]

Therefore, the purpose of this study was to evaluate the remineralizing and antibacterial effects of natural agents (propolis, hesperidin) versus synthetic agents (silver diamine fluoride) after the treatment of deep carious dentin at different time intervals. The study was conducted to accept or reject the null hypotheses that:

There is no difference between natural and synthetic agents in remineralization effect of carious dentin.

There is no difference between natural and synthetic agents as antibacterial agents.

Material and methods

All materials used in this research are included in Table 1.

All procedures were carried out by two individuals. The first individual is the primary investigator who was responsible for all clinical procedures and collection of samples, whereas the second individual was responsible for microbiological assessment and lab procedures.

Study design

This clinical study was conducted in the restorative dental clinic at the Faculty of Dental Medicine for Girls of Al-Azhar University, Cairo, Egypt. It was conducted between January 2019 and March 2020. The study was planned in accordance with the CONSORT 2010 criteria (Fig. 1). The Ethical Committee at the Faculty of Dental Medicine for Girls of Al-Azhar University approved the research protocol (REC-OP-21-05) and the trial was registered at ClinicalTrials.gov under the identification number NCT04145102.

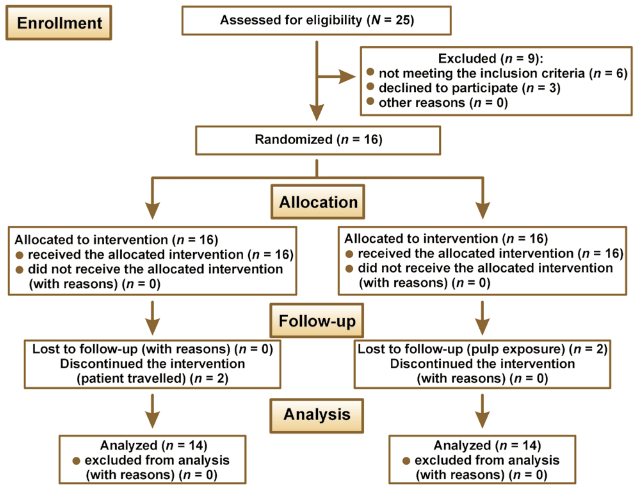

Fig. 1. CONSORT 2010 flow chart

Patient recruitment and eligibility criteria

Participants were recruited from the outpatient clinics of the Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt. Patients were enrolled in the study following the eligibility criteria presented in Table 2 after signing a fully descriptive informed consent.

Sample size calculation

According to Kabil et al 2018,[23] assuming an alpha (α) level of 0.05 (5%) and a beta (β) level of 0.20 (20%), i.e., a power of 80% and an effect size (f) of 0.595, the estimated sample size (*N*) was a total of 48 samples, i.e., 6 per group. The overall sample size was increased by 25% to 64 to account for possible dropouts, i.e., 8 samples per group. The G*Power v. 3.1.9.2 software was used to determine the sample size.

Grouping of the samples

A total of 64 human teeth (upper and lower, first and second molars) were selected for this study. Teeth were grouped into four main groups of 16 each, according to the treatment

Table 1. Materials used in the study

Substances	Brand name	Components	Manufacturer	Batch No.
Propolis extract	Bee propolis	flavonoid-rich propolis (2400 mg), zinc oxide (600 mg), polyethylene glycol 400 (1400 mg)	Imtenan Health Shop Company, Cairo, Egypt	FS011215
Hesperdin pure powder	Hesperdin	80% hesperidin, Hesperetin-7-O-rutinoside is a flavonoid extracted from citrus fruit	Sigma-Aldrich, St. Louis ,USA	SLBT3541
SDF with potassium iodide	Riva Star capsules	step 1 (the silver capsule): 30–35% silver fluoride and >60% ammonia solution step 2 (the green capsule): saturated potassium iodide (KI) solution	SDI Ltd., Bayswater, Australia	1115222
Mitis Salivarius agar			BD DIFCO™, Franklin Lakes, USA	2132229
Conventional glass ionomer	Riva Self Cure capsules	compartment 1: liquid, 20–30% acrylic acid homopolymer, 10–15% tartaric acid compartment 2: powder, 90–95% fluoroaluminosilicate glass, 5–10% acrylic acid homopolymer.	SDI Ltd., Bayswater, Australia	B1508072F

SDF – silver diamine fluoride.

Table 2. Selection criteria

Inclusion Criteria	Exclusion Criteria
Age: 18–50 years of both genders	Poor oral hygiene evident by rampant caries
According to the ADA caries risk assessment criteria for adults, at least one permanent molar with a deep carious lesion (class I cavity) in people with a medium or high caries risk	Pregnancy or breastfeeding
No pulpal involvement	Presence of chronic debilitating disease
Bitewing radiograph showing extension into inner 1/3 if dentin with a radiopaque film between the carious lesion and the pulp cavity.	Spontaneous pain greater than 15 seconds after sensitivity cold assessment that would indicate irrecoverable pulpitis, Internal or external resorption.
	Previous restorations or cracked enamel hypersensitivity
	Shallow or multi-surface carious lesions

agent applied (A): A1. propolis, A2. hesperidin, A3. SDF, and A4. control (no treatment). Each group was then divided into three subgroups according to the assessment time interval. Assessments were conducted at baseline which was obtained before treatment (B), after 6 weeks (B1), and after 12 weeks (B2) of applying the investigated materials.

Randomization, blinding, allocation sequences

Randomization was undertaken using an excel sheet with random numbers. A list of sequential numbers was created, in which each randomly assigned participant in this list was assigned a sequence number (ID) from 1 to 16, to be assigned to one of the four treatment groups. This trial was double blinded.

Treatment procedure

Preparation of the cavities and baseline samples

Pre-operative radiographs were taken using a digital X-ray machine (Kodak 2200; Kodak, Rochester, USA) with an imaging plate (digital sensor size 2; Dürr Dental, Bietigheim-Bissingen, Germany). Local anesthesia (Mepecaine- L) was administered with the infiltration technique and the nerve block technique for upper and lower arches respectively. Heavy sheet rubber dam isolation was applied, and class I cavities were prepared. Selective caries excavation technique was used to clean the pulpal floor using a spoon double ended excavator until leathery or firm dentin was reached as shown in Fig. 2.

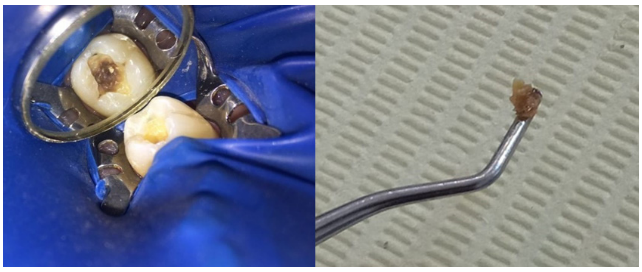

Fig. 2. Excavation of a deep layer of carious dentin

Samples of carious dentin were taken from the center of the carious lesion with the same-sized excavator for microbiological assessment. The samples were kept in sterile vials containing phosphate-buffered saline and delivered to the microbiology laboratory within two hours of extraction for processing.

Application of treatment agents

Each group received treatment following the manufacturer's instructions.

Group A1 was treated by covering the remaining carious lesions after cavity preparation by propolis extract as shown in Fig. 3. After preparation and toilet of the cavity the propolis extract was applied to the prepared floor by amalgam carrier, then condensed to cover the remaining carious lesion by condenser until a second sample could be taken depending on group allocation (i.e., group B1, 6 weeks; group B2 12 weeks).

Group A2 was treated with pure hesperidin powder mixed with distilled water at a 3:1 ratio to achieve a green like clay. The mix was applied using an amalgam carrier to the remaining carious lesion as shown in Fig. 4.

Group A3 was treated by SDF+KI (RIVA STAR, SDI, Bayswater, Australia) following the manufacturer's instructions. The capsules are color coded; the SDF is gray colored, and a brush was used to apply the material from the capsule and onto the cavity floor as shown in Fig. 5. The second step involved using potassium iodide (KI) which is green coded and applied until a creamy white precipitate turned clear followed by oil free drying.

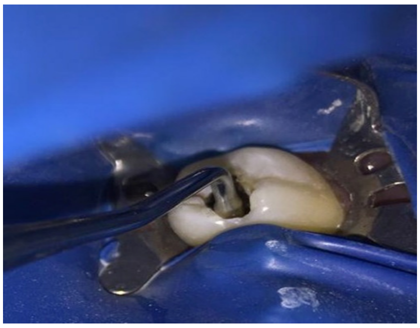

Fig. 3. Application of propolis

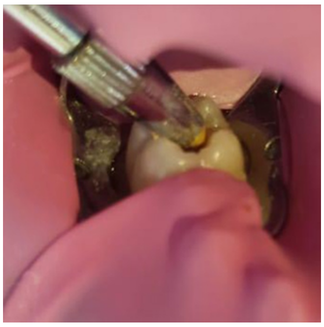

Fig. 4. Application of hesperidin

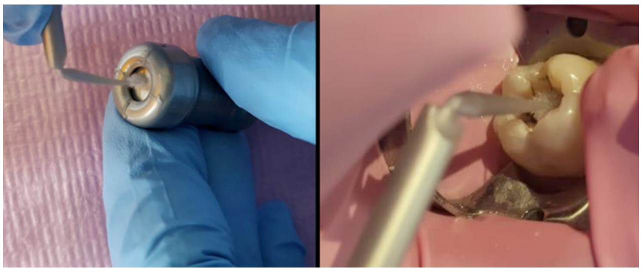

Fig. 5. Application of silver diamine fluoride (SDF)

Restorative procedures

Stepwise excavation technique included two treatment visits. In the first visit, initial baseline digital radiograph was carried out prior to cavity preparation. A cavity was then prepared, and caries removed before cavity conditioning was done using a micro brush in a rubbing motion to all surfaces except the pulpal floor. Conditioner was rinsed using water for 10 s and moist dentin was maintained by eliminating excess water. This was followed by tested material's application. Finally, a highly viscous glass ionomer cement glass ionomer (GIC) restoration material was applied to seal the cavities. Excess material was removed by a sharp explorer and allowed to sit for 6 minutes. Finishing by high-speed finishing stones was performed. Riva coat was applied and cured with LED intensity of 1200 mW/cm^2 for 20 s. Another digital radiograph was taken and labeled baseline radiograph. Patients were dismissed with restoration retained until their second visit for recovery of the second dentin sample and second radiograph. Patients were instructed to maintain proper oral hygiene and to appear for all follow-up appointments.

For the second visit, the glass ionomer cement GIC was removed using a diamond bur with coolant and only the last layer of GIC was removed using a sharp spoon excavator. The last layer of GIC was identified with the guidance of the digital x-ray film taken on the first visit. Also, remaining carious dentin was mostly darker in color and created a dark shadow apparent beneath the last layer of the GIC. The hand instrument was used to preserve the most superficial layer of dentin for bacterial sample collection. A second sample was taken and handled as previously described. The cavity was then filled with highly viscous GIC base to the level of the dentin–enamel junction (DEJ), and then a final restoration was used. The timing of the second visit depended on which group the patient was allocated to. For B1 patients would come for a second image, sample collection, final restoration after 6 weeks, and then a final visit would occur at 12 weeks for a third image. On the other hand, B2 (12 weeks) patients would come after 6 weeks for imaging, and then again after 12 weeks for sample collection and third imaging Fig. 6.

Final restoration was done with composite resin material using a selective etching technique utilizing 35% phosphoric acid on enamel surface for 15 s, rinsed with water for 15 s, gentle air water/oil-free for 5 s, blot-drying any moisture by absorbent tissue. A micro brush was used in a rubbing motion to apply a single layer of universal adhesive for 10 s, air drying for 5 s, and then light cured for 20 s. Shade selection was done, and 2 mm incremental packing of composite cured for 60 s was performed. Finishing and polishing was then conducted.

Assessment of remineralization

To assess the dentin remineralization, a digital X-ray machine (Kodak 2200) was used with DBSWin software at baseline, 6 weeks and 12 weeks, using a size 2 digital sensor and posterior parallel kit for image standardization that has 2 ends: one for X-ray cones and the other for the sensor. Then, the sensor was removed and scanning was performed with the use of Dürr Dental Vista Scan. The scans were stored for analysis.

Measurements in each sample were fixed for all samples at assessment intervals; 2 lines were drawn, one along the cementoenamel junction (CEJ) for reference and a second line parallel to it at the bottom of the cavity. The distance between the 2 lines was standardized for each tooth and measured by a vertical line connecting them. The DBSWin software was then used to determine the length in pixels and fix it for each sample. Three points on this line (at its start, middle, and end) were determined. The intensity of these points was recorded at each follow-up visit.

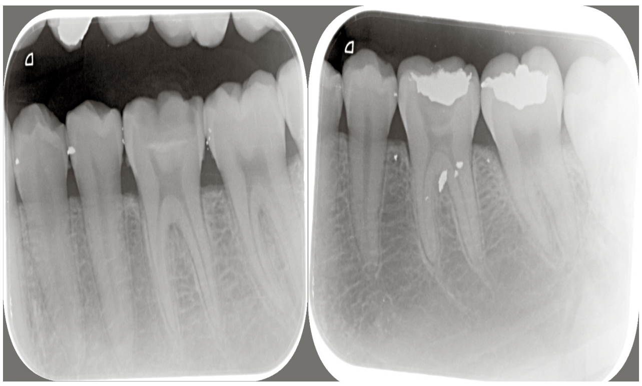

Fig. 6. Radiographic images before and after treatment with the propolis agent

Bacterial assessment and processing of the dentin samples

Samples were obtained from soft dentin carious lesions of permanent teeth of adult patients and transferred into a sterile 2 mL CryoTube™ (Thermo Fisher Scientific, Waltham, USA) containing 1 mL of phosphate buffer saline (PBS), which then transported to the microbiology laboratory for analysis. Bacterial dispersion into the media from carious dentin samples was achieved by vortexing the samples for 30 s.

Isolation and identification of *Streptococcus mutans*

Using double ended sterile calibrated loops, 10 μL of the diluted samples were uniformly spread on the surface of mitis salivarius bacitracin (MSB) agar plates. The plates were sealed and incubated anaerobically by using a gas pack supplied in an anaerobic jar. Colonies of *Streptococcus mutans* were identified based on their unique morphology on MSB, with elevated, convex, opaque colonies of deep blue color with rough edge and particulate frosted glass aspect classified as *Streptococcus mutans*. Under microscopic examination the *Streptococcus mutans* appeared as Gram positive cocci arranged in chains. Biochemical tests such as the catalase test, hemolysis on blood agar and positive fermentation with sorbitol and mannitol were performed for verification of *Streptococcus mutans*.[24]

Statistical analysis

Numerical data were investigated with the Kolmogorov–Smirnov and Shapiro–Wilk tests. The bacterial count data was log transformed. The results displayed a parametric distribution; thus, they were described as mean and standard deviation ($M \pm SD$). The two-way analysis of variance (ANOVA) was used to investigate the impact of different variables and their associations. The one-way ANOVA with Tukey's post hoc test was used to investigate the main effects. The significance level was set at $p \leq 0.05$ for all tests. Statistical analysis was performed with the use of IBM SPSS Statistics for Windows, v. 25.0 (IBM Corp., Armonk, USA).

Results

Radiodensity assessment results

The comparison of the mean percentage changes in dentin radiodensity for different agents within each time interval (ΔT) is presented in Table 3 and Fig. 7.

Baseline to 6 weeks (ΔT1)

Results of the ANOVA test showed that there was a significant difference between values of different groups ($p < 0.001$). Silver diamine fluoride (A3) had the highest (83.05 ±8.78) value of percent change of dentin radiographic density followed by control group (A4) (29.15 ±5.23) then propolis group (A1) (16.17 ±2.79), with the hesperidin group (A2) (14.22 ±2.53) having the lowest value. Tukey's post hoc test comparison showed that the value for SDF group (A3) group was significantly different from that of the other materials. It also showed that hesperidin group (A2) was significantly different from that of other groups except propolis (A1) ($p < 0.001$).

Baseline to 12 weeks (ΔT2)

Results of the ANOVA test showed that there was a significant difference between values of different groups ($p < 0.001$). SDF group (A3) had the highest (110.92 ±8.55)

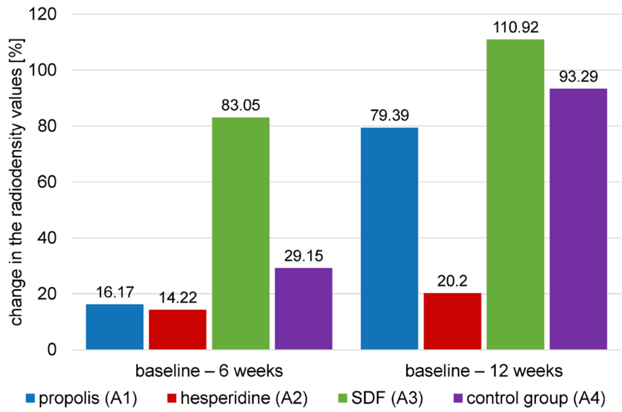

Fig. 7. Bar chart illustrating the percentage changes in the mean values of dentin radiodensity for different materials at different time intervals

Table 3. Percentage changes in the mean values of dentin radiodensity for different materials within each time interval (ΔT)

Time interval (ΔT)	Treatment agents				*f*-value	*p*-value
	propolis (A1)	hesperdine (A2)	SDF (A3)	control group (A4)		
Baseline to 6 weeks (ΔT1)	16.17 ±2.79[c]	14.22 ±2.53[c]	83.05 ±8.78[a]	29.15 ±5.23[b]	210.72	<0.001*
Baseline to 12 weeks (ΔT2)	79.39 ±9.68[c]	20.20 ±5.53[d]	110.92 ±8.55[a]	93.29 ±7.57[b]	188.87	<0.001*

Data presented as mean ± standard deviation ($M \pm SD$). Different superscript letters indicate a statistically significant difference within the same horizontal row; * statistically significant ($p \leq 0.05$).

value of percent change of dentin radiographic density, followed by the control group (A4) (93.29 ±7.57), then the propolis group(A1) (79.39 ±9.68), with the hesperidin group (A2) having the lowest value (20.20 ±5.53). Tukey's post hoc test comparison showed that the values of all groups were significantly different from each other ($p < 0.001$).

Microbiological results

The comparison of the percentage changes in the log transformed mean values for bacterial count (CFU/mL) of different treatment agents within different time intervals is presented in Table 4 and Fig. 8.

Baseline to 6 weeks (ΔT1)

Results of ANOVA test showed that there was a significant difference between the values of different groups ($p < 0.001$). The highest percentage change of the log transformed mean values for bacterial count (CFU/mL) was found in the SDF group (A3) (76.64 ±6.75) group, followed by the control group (A4) (50.56 ±6.47), then the propolis group (A1) (43.82 ±6.56), and finally the hesperidin (A2) group (13.33 ±4.60). Tukey's post hoc test comparison showed that the percentage change in the log transformed mean values for bacterial count (CFU/mL) of SDF group (A3) was significantly higher than all the other groups. It also showed that hesperidin group (A2) was significantly lower than all other groups except the propolis group (A1). Finally, the propolis group (A1) and the control group (A4) were not significantly different from each other ($p = 0.001$).

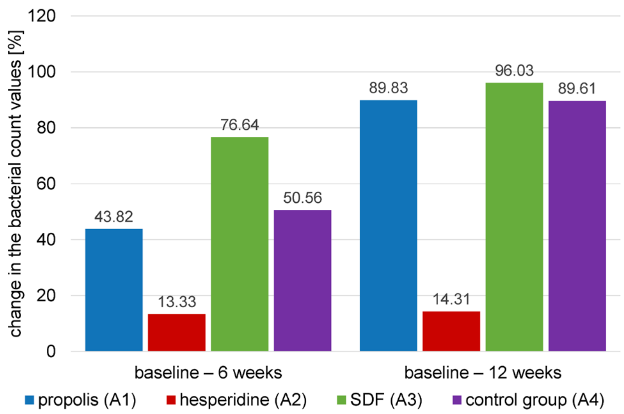

Fig. 8. Bar chart showing the percentage changes in the mean values for the bacterial count for different treatment agents at different time intervals

Baseline to 12 weeks (ΔT2)

Results of the ANOVA test showed that there was a significant difference between values of different groups ($p < 0.001$). The highest percentage change in the log transformed mean values for bacterial count (CFU/mL) was found in the SDF group (A3) (96.03 ±2.68) group, followed by the propolis group (A1) (89.83 ±6.14), the control group (A4) (89.61 ±7.80), and finally the hesperidin group (A2) (14.31 ±4.03). Tukey's post hoc test results showed that hesperidin group (A2) had a significantly lower value than the other groups ($p < 0.001$).

Discussion

This work was devised to evaluate the remineralizing and antibacterial effect of propolis and hesperidin versus that of silver diamine fluoride in deep carious dentinal lesion. Subjects were randomized to different treatment groups to ensure the internal validity of comparisons between novel treatments.[25]

The remineralizing and antibacterial effects of these compounds was evaluated at two separate intervals (6 and 12 weeks). This enabled us to monitor the initial mineral gaining and antibacterial activity in the four test groups, and remineralization of infected dentin remaining after stepwise excavation, respectively. These time intervals were picked to minimize the risk of deterioration of the short-term temporary restoration and patient dropout.[13]

The pixel grey measurement in digitized radiograph method was used to assess remineralization. A study by Mittal et al. reported that the average pixel grey value can be used to quantitatively monitor caries remineralization, this is based on remineralization being a slower process than demineralization.[26]

Colony forming unit (CFU) is a measure of viable bacterial cells. For convenience, liquid data are expressed in CFU/mL (colony-forming units per milliliter) and solid findings in CFU/g (colony-forming units per gram). This method is useful to determine the microbiological load and magnitude of infection in any samples.[27] After re-

Table 4. Percentage changes in the log transformed values for the bacterial count for different treatment agents within each time interval (ΔT)

Time interval (ΔT)	Treatment agents				*f*-value	*p*-value
	propolis (A1)	hesperdine (A2)	SDF (A3)	control group (A4)		
Baseline to 6 weeks (ΔT1)	43.82 ±6.56[b]	13.33 ±4.60[c]	76.64 ±6.75[a]	50.56 ±6.47[b]	100.49	<0.001*
Baseline to 12 weeks (ΔT2)	89.83 ±6.14[a]	14.31 ±4.03[b]	96.03 ±2.68[a]	89.61 ±7.80[a]	278.94	<0.001*

Data presented as *M* ±*SD*. Different superscript letters indicate a statistically significant difference within the same horizontal row; * statistically significant ($p \leq 0.05$).

moval of the superficial dentin, samples of carious dentin were taken, to ensure that microorganisms were from the body of the lesion and not dental plaque.[28]

Propolis (bee glue) is one of many herbal products that possess antibacterial properties. Propolis promotes dental pulp regeneration by preventing microbial infection, inflammation, and pulp necrosis.[29] The antimicrobial activity of propolis is derived from its flavonoids, phenolic acids, and phenolic acid esters which can interfere with bacterial cell membranes and cytoplasm, and suppress DNA synthesis.[30] Additionally, it promotes stem cells to create an effective tubular dentin.[29] Propolis was selected in this study as it is safe for human application, inexpensive, and accessible.

Similarly, hesperidin, a natural flavonoid, is the most active compound of orange fruit and is found in the peel and pulp of the fruit. It consists of a glycogen sugar part bound to the non-sugar part called aglycone. It was used in this study to induce remineralization, antibacterial activity, and arrest active carious dentin.[31] Hesperidin was mixed with distilled water due to its higher solubility, which is important for the therapeutic action of the pure powder due to the presence of hydroxyl rings.[31] In this clinical trial, remaining carious dentin was capped by natural materials during the whole assessment period. This is supported by the findings of a previous study,[32] which suggested that keeping propolis in the mouth produced a greater antibacterial effect, resulting in a considerable reduction in the extent of secondary caries.

Silver diamine fluoride (SDF) is another treatment for slowing the course of dental caries. It achieves this through the antibacterial action of silver halting bacterial growth and the acid resistant fluorapatite surface formed by fluoride.[33] Silver ions are bactericidal metal cations that limit microbial growth by suppressing and impeding microbial polysaccharide production through the inhibition of glycosyltransferase enzymes. Unfortunately, the silver content of SDF has some undesired side effects; SDF causes discoloration and an unpleasant metallic taste.[34]

Self-cured highly viscous GIC were used to restore all tested material and were placed over deep carious lesions due to their high sealing ability which is an important factor for the success of the therapy. Additionally, GIC can release fluoride and chemically bond to moist tissue.[35]

Radio density results revealed that treatment of active carious dentin with different agents (propolis, hesperidin and SDF) had significantly higher mean mineral dentin density values at the different assessment intervals. After 6 weeks, the results showed more mineral density of carious dentin in the SDF group than in other groups (control, propolis, and hesperidin). While an acid attack, it steadily discharges fluoride to constrain pH and form acid-resistant fluorapatite.[31] This is important given the involvement of silver ions, high fluoride content, and chemical reaction with the residual hydroxyapatite in carious dentin. One of the reactions' byproducts is calcium fluoride (CaF_2), which behaves as a fluoride storage.[36,37]

Fluoride ion is more similar to the crystal structure of hydroxyapatite than the hydroxyl group is. As a result, fluoridated apatite has a lower solubility than fluoride-free apatite. Consequently, it promotes remineralization by precipitating calcium and phosphate ions, as well as by increasing the precipitation of fluoride apatite above the critical pH.[38,39] In addition, it can protect the organic matrix of dentin in two different ways. First, mineral crystals can protect collagen molecules by binding to calcium binding sites, resulting in less depleted collagen fibers. Second, fluoride ion is a powerful inhibitor of matrix metalloproteinases 2, 8, and 9. Fluoride has also been shown to inhibit cathepsins B and K which are required for MMP activation.[39]

Aside from the fluoride ion mechanism of action, SDF is an alkaline solution with acidity 10.[39] This state promotes the association of covalent bonds between phosphorus ions and collagen molecules, which are required for collagen protection.[37]

The control group (restored only with GI) showed higher mineral carious dentin density over 6 weeks compared to baseline than other natural agents, but it was less effective than SDF. This is directly attributable to the release of fluorine from the glass ionomer cement. Moreover, sealing the cavities with glass ionomer or another restorative material may lead to arresting of caries which is attributed to the absence of bacteria.[40]

While both hesperidin and propolis showed remineralizing effects on carious dentin, the results from these treatments did not differ from each other. After 6 weeks the propolis group had increased mineral density of carious dentin mainly due to flavonoids present in it that can induce reparative dentin through upregulation of growth factors (TGF-$\beta 1$) which interact with the extracellular matrix resulting in collagen formation.[41,42] Furthermore, flavonoids can inhibit microorganisms by denaturing proteins and nucleic acids. Hindering of bacteria will decrease demineralization and help in mineral precipitation. Also, the presence of vitamin B complex, pro-vitamin A, arginine, and minerals such as copper, iron, zinc, and bioflavonoids can induce formation of new hydroxyapatite crystals.[43,44]

Meanwhile, in the hesperidin group, a non-significant difference in mineral density value of carious dentin and remineralizing effect on carious dentin was observed between the 6 and 12-week periods. This may be the result of the slightly acidic nature of hesperidin, which is obtained from citrus fruits. This low pH media results in low calcium and phosphorus ions participation which reduces remineralizing and the antibacterial effect.[31]

A study found that the application of standardized propolis extract as a pulpotomy medication caused the formation of a partial mineralized tissue barrier after 21 days, and a complete calcified bridge after 42 days.[45] Meanwhile, SDF showed the same action in less time with a high amount of fluoride and silver halt active carious lesions and provide antibacterial action. Our findings agree with another study,[41] which compared the remineralizing and antibacterial effect of SDF and propolis. It was found that SDF had superior antibacterial and remineralizing ability, which was attributed to the concentration of active ingredients in SDF that reach up to 38%, as opposed to propolis fluoride which only contains 10% active material. Our clinical trial results showed a decrease in bacterial total counts over the different assessment periods compared to baseline data in active carious dentin lesions treated with natural or synthetic agents.

After 6 weeks, the SDF group had the greatest reduction in bacterial counts, followed by the control and propolis groups respectively. SDF likely had the greatest antibacterial effect because of the presence of silver and fluoride ions. Antimicrobial activity of silver ions can be attributed to several factors; when silver ions react with the thiol group of enzymes, they deactivate the enzymes which then cause bacterial death.[46,47] These silver ions could also interact with bacterial cell DNA, which can cause mutations in the DNA and bacterial cell death. Furthermore, silver binds to the anionic parts of the bacterial cell membrane which can cause bacterial death. Silver can also create a protein-metallic combination with amino acids, which then can concentrate within the bacterial cell rendering bacterial DNA and RNA inactive.[15] Our data is in line with an earlier study,[48] which found that SDF's effectiveness in arresting pre-existing dentin caries and preventing new caries was due to the synergistic effect of silver and fluoride ions in inhibiting cariogenic bacterial growth, remineralization, and organic matrix protection.

Restoration of active carious dentin with GIC only, without any treatment, showed a significant decrease in the total bacterial count. This could be due to the fluoride released by conventional GIC restoration. Fluoride ions can promote remineralization, inhibit cariogenic bacterial growth and enhance the calcification of demineralized dentin after curettage.[49]

Total bacterial count in active carious lesions was decreased when using propolis. Propolis can prevent bacterial proliferation and inhibit bacterial protein synthesis which can cause partial lysis.[46] Propolis constituents such as pinocembrin and naringenin also possess antibacterial activity.[47]

Silver ions can inactivate and interfere with bacterial growth by inactivating glycosyltransferase enzymes, which are needed for glucan synthesis. Glucan is required for bacteria sucrose-dependent adhesion to tooth surfaces.[48–50]

In the present work, there was a significant difference between the natural agent propolis and the synthetic agent SDF, which was attributed to the natural agents needing more time. This may be due to the concentration of active ingredients being lower in the natural agent.[51] Propolis and hesperidin are natural materials which may mean that they have increased safety and fewer side effects compared with synthetic products. Therefore more clinical trials are required to investigate differences in the efficacy between natural and synthetic materials.

Finally, in the present study, the first null hypothesis that there is no difference between natural and synthetic agents on the remineralizing potential of carious dentin was rejected. We found that there was a statistically significant difference between each material. In addition, the second null hypothesis that there is no difference between natural and synthetic agents as antibacterial agents was partially accepted as we found that there was a statistically significant higher percent change of bacterial count for propolis and SDF compared with hesperidin.

Conclusions

Propolis may be a promising substitute for synthetic remineralizing and antibacterial agents. Silver diamine fluoride has powerful antibacterial and remineralizing effect. Marginal seal seems to be a crucial factor in the management of deep carious dentin.

Recommendations

Further clinical trials are required to evaluate the pulpal outcome against the applied materials. Further clinical trials also are required to investigate the clinical performance of other natural materials.

Trial registration

The trial was registered at ClinicalTrials.gov under the identification number NCT04145102.

Ethics approval and consent to participate

The research protocol was approved by the Ethical Committee at the Faculty of Dental Medicine for Girls of Al-Azhar University (REC-OP-21-05). Written informed consent was obtained from all participants prior to 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.

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Original paper

Comparison of periodontal indices, DMFT, xerostomia, hyposalivation and oral health-related quality of life in Sjögren's syndrome patients versus healthy individuals: A case–control study

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Abstract

Background. Sjögren's syndrome (SS) is a common systemic autoimmune disease that affects oral health, and consequently oral health-related quality of life (OHRQoL) due to the involvement of exocrine glands.

Objectives. The present study aimed to evaluate the oral health-related quality of life and oral health indicators in patients with SS in comparison with healthy individuals.

Material and methods. In the case and control groups (45 patients and 45 healthy individuals), questions about demographic data, other systemic disorders, medications, the years of infection, xerostomia, as well as inquiries about the quality of life (Oral Health Impact Profile-14 – OHIP-14) were asked. The patients were evaluated clinically, and oral health indicators, including the plaque index (PI), the gingival index (GI), the sulcus bleeding index (SBI), and the number of decayed, missing and filled teeth (DMFT) were assessed on the Ramfjord teeth. Unstimulated saliva samples from both groups were obtained and weighed. The data was analyzed using IBM SPSS Statistics for Windows, v. 24.0. Quantitative variables were compared between the case and control groups with the use of the independent t test or their non-parametric equivalent (the Mann–Whitney test).

Results. The comparison of the quantitative variables between the study groups showed a statistically significant difference in the OHRQoL scores ($p = 0.037$) and the unstimulated saliva flow rate ($p = 0.002$) between the case and control groups. Also, there was a statistically significant difference in the DMFT index between patients with primary and secondary SS in the case group ($p = 0.048$).

Conclusions. The lower OHRQoL of patients with SS requires more attention and follow-up to solve periodontal and dental problems in this group of patients.

Keywords: oral health-related quality of life, Sjögren's syndrome, plaque index, gingival index

Introduction

Sjogren's syndrome (SS) is an autoimmune disease of the exocrine glands that mainly affects the salivary and lacrimal glands.[1] This disease affects 3–4% of the adult population and primarily affects middle-aged to older women. The prevalence of this disease in the European population varies from 0.05 to 1%.[2,3] The condition can be divided into primary and secondary SS.[4] It can be present alone (primary SS, pSS) or in association with other systemic autoimmune disorders such as lupus, rheumatoid arthritis, and connective tissue diseases (secondary SS, sSS). The clinical presentation of this disease is characterized by dryness of the entire mucosa. Symptoms may range from localized outcomes of the exocrine glands to systemic complications such as vasculitis.[5] Although the etiology of this disease is still unknown, the current hypothesis supports an autoimmune reaction within the exocrine glands, which triggers the uptake of salivary gland and lacrimal cell nuclear factors. Over the years, this pathological process leads to lymph node destruction and disruption or even total loss of saliva and tears.[6] SS can affect the overall quality of life related to general health.[1] Fatigue, pain, and systemic manifestations are the predominant complications of this disease that affects the health-related quality of life.[7] Quality of life is a multidimensional and broad concept that means a person's understanding of their life situation according to culture and value systems, expectations, standards, and life experiences that affect the state of physical, mental, social communication, and personal beliefs.[8] Quality of life is a valuable indicator used to measure health status in research.[9] Oral health-related quality of life (OHRQoL) includes a part of the quality of life affected explicitly by oral health.[10]

Decreased salivation in patients with pSS can have a significant negative effect on oral health.[11] Oral symptoms of SS are mainly due to decreased salivary flow.[12] Xerostomia can be severe and can lead to discomfort and problems with speech, eating, swallowing, changes in taste, candidiasis of the mouth, tooth decay, and periodontal problems.[13] In these patients, oral issues play a critical role in OHRQoL.[14] Numerous studies have linked decreased salivation to oral problems.[15,16] A few studies have reported taste and smell disorders in patients with early SS.[17] However, the effect of oral symptoms of SS on OHRQoL is not yet fully understood.[18] Health interventions are increasingly being used to assess the impacts of oral disorders.[19] A comprehensive approach to measuring OHRQoL combines the use of general and specific oral criteria with specific conditions.[20] Oral Health Impact Profile (OHIP) is currently one of the most comprehensive measures of the effect of oral status on health-related quality of life.[21] Assessing patient-based health status is essential to measuring health. Oral diseases are prevalent and have physical, social, economic, and psychological consequences on patients. Many of these patients' quality of life is impaired, and various aspects of their lives, such as chewing food and speech, can be affected.[22] The OHIP was developed by Slade and Spencer in 1994 and is an advanced OHRQoL tool that is used internationally.[23] The main OHIP consists of 49 items that can be long and time-consuming. In 1997, Slade developed a short form with 14 questions called the OHIP-14 that was found to have good reliability, accuracy, and credibility.[24] These fourteen items of the OHIP fall into seven aspects: functional limitations, physical pain, mental discomfort, physical disability, mental disability, social disability, and disability.[23]

Therefore, considering the significant effects of SS on general health and oral health, the lack of a similar study in Iran, and to measure of some variables that have not been studied so far, this study aims to evaluate the OHRQoL of patients with SS referred to the Touba Clinic in Sari in 2020. The null hypothesis is that there is a difference between SS patients and healthy individuals when looking at periodontal indices, Decayed, Missing, and Filled Teeth (DMFT) index, xerostomia, hyposalivation, and OHRQoL.

Material and methods

The present study is a case-control study of patients referred to the Touba Clinic in Sari, India, in 2020. The Research Ethics Committee of Mazandaran University of Medical Sciences approved this study (Ethics Code: IR.MAZUMS.REC.1398.6148). The sample size was estimated using the results of a study by Meijer et al.[4] In their study, the mean and standard deviations were 59.2 ±26.0 in the case group and 74.8 ±25.8 in the control group, respectively. Considering these results, a 95% confidence level, an 80% test power, two test ranges, and using the comparison formula between means and using the G*power software, the sample size was estimated to be 90 people (45 people in the case group and 45 in the control group).

The inclusion criteria included people with primary or secondary SS diagnosed using the American-European Consensus Group criteria[5] with an age range of 30–80 years who had at least 20 teeth in their mouth and no history of periodontal treatment in the past three months. The exclusion criteria included patients who are illiterates, patients who were unable to complete the questionnaire, and patients with chronic gastrointestinal diseases, organ transplantation, diabetes mellitus, infectious diseases (hepatitis, HIV), seizure disorder or neuropathy, heart failure, drug users, pregnant women, smokers, and alcoholics.[25] The control group was selected from patients referred to the Sari Dental School clinic. In addition to the mentioned inclusion and exclusion criteria, age and gender were considered for the case group. Also, the control group was negative for diabetes based on fasting blood sugar (FBS) test results in the month before the start of the study.

Data collection

The purpose of the research and its steps were initially explained to all participants, and after obtaining written consent, patients were assured that their information would remain confidential. This study's data collection method used a question-and-answer session, a questionnaire, patient records, and a clinical oral examination by a periodontist. Demographic information for the patients (age, gender) and information related to the patient (duration of illness, medications) were recorded in the patient's files. To determine the presence of xerostomia, patients were asked 9 questions, and those who answered 9 to 5 questions positively were diagnosed with xerostomia.[25]

Unstimulated saliva flow rate

To assess the unstimulated saliva flow rate (USFR), individuals were asked to avoid drinking, eating, and smoking for at least 2 h before sampling. Patients with partial dentures were asked to remove them from their mouths. While collecting unstimulated saliva samples, individuals were asked to sit and reduce their mouth movement and not swallow or suck. Saliva was then collected from the floor of their mouths for 60 s. They were then asked to spit it in a pre-weighed tube for five consecutive minutes. The saliva samples were stored in the freezer at −70°C until assayed. The amount of unstimulated saliva was measured by the weighting method and expressed in milliliters per minute. A USFR <0.1 mL/min was considered to indicate hyposalvation.[26]

Oral health-related quality of life

To assess OHRQoL in this study, the OHIP-14 questionnaire was used, and the validity and reliability of the Persian version were confirmed[27] and included the seven subgroups: functional limitation, physical pain, mental discomfort, physical disability, mental disability, social disability, and disability. Each subgroup consisted of 2 questions. Two methods were considered to evaluate the responses. The additive[17] method in which test options were scored as never = 0, seldom = 1, sometimes = 2, most often = 3, in the majority of cases = 4. The OHIP-14 scores could range between 0 and 56, and the lower the score, the better the quality of life for the patient. In another evaluation method called simple count (SC), the score given for "never" and "seldom" options is zero, and the score given for sometimes, most often, and in the majority of cases is one. This method was used because some people do not understand the real difference between the questionnaire options. The OHIP-14 score ranges from zero to 14 using the SC method, and again the lower the score, the higher quality of life in the patient.[27]

Periodontal indices

Finally, the patients were clinically examined by a periodontist. The DMFT, the plaque index (PI), the gingival index (GI), and the sulcus bleeding index (SBI) were performed on the Ramfjord teeth (teeth numbers 3, 9, 12, 19, 25, and 28), and if one of these teeth were missing, the lateral tooth was used.[28]

Statistical analysis

To analyze the data, all the recorded information was entered into the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). The normality of quantitative variables was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The variables were described as mean and standard deviation ($M \pm SD$), minimum and maximum, and as number and percentage (n (%)). The normally distributed variables were compared using the independent t tests, whereas abnormal parameters were compared using a non-parametric comparative test (the Mann–Whitney test), and for multiple groups, the non-parametric Kruskal–Wallis H comparison test was performed. To examine the correlation relationships, normal data utilized the Pearson correlation method, whereas abnormal data used the Spearman method. Also, for the comparison of variables between the groups, the χ^2 test and Fisher's exact test were used. Finally, to rank and prioritize abnormal data, the Friedman ranking method was used. The criterion in which all cases reached statistical significance was a p-value <0.05, except for the normality and equality of variance.

After obtaining a license from the esteemed vice-chancellor of research at the university, the ethics committee began the process in Sari using the ethics code IR.MAZUMS.REC.1398.488 and obtained approval from the competent authorities. This research started by obtaining patient consent after explaining the potential advantages and disadvantages of the study, the goals of the project, the voluntary nature of the study, the possibility of leaving the study at any time, and the process of keeping the patients' information and identity confidential.

Results

A total of 90 people (45 in the case group and 45 in the control group) participated in this study. In the case group, 19 patients (42%) had pSS, and 26 patients (58%) had sSS (22 patients with rheumatoid arthritis and 4 patients with scleroderma). There were 40 women (88%) and 5 men (12%) in both groups. The mean age of the case group was 50.62 ±7.82 years, and the control group was 50.62 ±7.82 years, which had a similar distribution that was not statistically significant (p = 0.999). The mean age in the case group with pSS was 47.68 ±8.42 years. The case group with sSS was 52.77 ±6.73 years, which was not statistically significant based on a non-parametric comparison test (Mann–Whitney; $p = 0.373$). The disease duration in the case group varied from 4 months to 20 years. The period of the disease had a significant relationship with the patient's age ($p = 0.027$) but had no statistical association with any other measured indicators. It should be noted that in the case group, 17 people (38%, 8 people with pSS, and 9 people with sSS), and in the control group, 6 people (13%) had hyposalivation. Examination of the 9-item xerostomia questionnaire revealed that 20 patients (45%) in the case group (8 patients with pSS and 9 patients with sSS) and only 4 patients (90%) in the control group had xerostomia.

The results of the analyzed quantitative variables in this study show that the mean DMFT index in the case group was 10.29 ±4.70. In the control group, it was 10.24 ±4.78, which was not statistically significant ($p = 0.965$). PI, GI, and SBI were also examined in the case and control groups, and the results showed that the mean PI in the case group was 1.33 ±0.44 and 1.37 ±0.39 in the control group, which was not significantly different ($p = 0.646$). The mean GI in the case group was 6.93 ±1.43 and 7.67 ±1.87 in the control group, which was not significantly different ($p = 0.206$). Also, the mean SBI in the case group was 0.93 ±1.42 and 1.53 ±1.77 in the control group, which was not statistically significant despite the apparent difference ($p = 0.210$). Saliva samples collected from the two groups after weighing showed that the mean USFR per minute in the case group was 0.93 ±0.99. The USFR per minute in the control group was 1.36 ±0.99, in which the difference was statistically significant ($p = 0.002$). It should be noted that 17 patients in the case group (38%) and 6 in the control group (13%) had hyposalivation (Table 1).

Table 2 summarizes the results of the correlation relationships between the quantitative variables. These results show that in the case group, there were significant correlations between PI with GI and SBI with coefficients of $r = 0.397$ and $r = 0.305$, respectively. There is also a stronger positive correlation between GI and SBI ($r = 0.89$).

Examination of Pearson correlations in the control group also shows that there is a significant and negative correlation between patient age and USFR ($r = 0.40$). There is also a relationship between age and PI ($r = 0.38$). There is a significant correlation ($p = 0.32$) between PI

Table 1. Statistical comparison of the studied quantitative variables in the case and control groups

Variables	Case group			Control group			p-value
	n	*M* ±*SD*	range	*n*	*M* ±*SD*	range	
ADD_OHIP14	45	19.56 ±12.46	0–50	45	14.42 ±10.46	0–42	0.037*
SC_OHIP14	45	6.60 ±4.02	0–14	45	4.71 ±3.81	0–13	0.025*
Age [years]	45	50.62 ±7.82	32–70	45	50.62 ±7.82	32–70	0.999
PI	45	1.33 ±0.44	1–3	45	1.37 ±0.39	1–3	0.646
GI	45	1.17 ±0.25	1–3	45	1.27 ±0.31	1–3	0.206
SBI	45	0.16 ±0.24	0–1	45	0.25 ±0.30	0–1	0.210
DMFT	45	10.29 ±4.70	2–21	45	10.24 ±4.78	1–21	0.965
USFR	45	0.19 ±0.20	0.00–4.18	45	0.27 ±0.20	0.14–4.54	0.002**

M – mean; *SD* – standard deviation; PI – plaque index; GI – gingival index; SBI – sulcus bleeding index; DMFT – number of decayed, missing and filled teeth; USFR – unstimulated saliva flow rate; * statistically significant ($p < 0.05$); ** statistically significant ($p < 0.015$).

Table 2. Correlation relationships between the quantitative variables in both study groups

Quantitative research variables	*n*	Statistical index	Case group – quantitative research variables					Control group – quantitative research variables				
			USFR	DMFT	SBI	GI	PI	USFR	DMFT	SBI	GI	PI
Age	45	correlation coefficient	−0.098	−0.075	0.081	0.233	0.119	−0.398**	0.224	−0.001	0.074	0.383**
		p-value	0.523	0.624	0.597	0.123	0.435	0.007	0.139	0.995	0.630	0.009
PI	45	correlation coefficient	−0.244	0.210	0.305*	0.397**	–	−0.364*	0.082	0.260	0.325*	–
		p-value	0.106	0.166	0.042	0.007	–	0.014	0.592	0.084	0.029	–
GI	45	correlation coefficient	−0.075	0.181	0.892**	–	–	−0.176	0.130	0.931**	–	–
		p-value	0.626	0.235	0.000	–	–	0.248	0.393	0.000	–	–
SBI	45	correlation coefficient	−0.075	0.181	–	–	–	−0.169	0.139	–	–	–
		p-value	0.626	0.235	–	–	–	0.260	0.362	–	–	–
DMFT	45	correlation coefficient	0.060	–	–	–	–	0.155	–	–	–	–
		p-value	0.695	–	–	–	–	0.310	–	–	–	–

* statistically significant ($p < 0.05$); ** statistically significant ($p < 0.015$).

and GI with a 95% accuracy. As in the case group, there is a strong correlation (*r* = 0.93) between the GI and SBI variables (Table 2).

In the study of OHRQoL as a dependent variable with the independent quantitative variables using Pearson correlation coefficient analysis, we observed that ADD_OHIP-14 and SC-OHIP in the case group had a positive and significant correlation with PI (*r* = 0.31 and *r* = 0.35, respectively). But in the control group, this correlation was not statistically significant. Other quantitative data, such as GI, SBI, and DMFT did not show a significant relationship with oral quality of life variables collected in the questionnaire (Table 3).

Comparative evaluation of patients with primary and secondary SS

Quantitative indices in patients with primary and secondary SS

The analyzed results of SS patients in the two groups of pSS and sSS in Table 4 showed that the mean ADD_OHIP-14 in pSS patients was 21.00 ±10.30. In sSS, it was 18.50 ±13.93, and the mean SC_OHIP14 in pSS was 7.21 ±1.08. In sSS, it was 6.15 ±4.01, which did not show a statistically significant difference (*p* = 0.493 and *p* = 0.392, respectively). Also, PI, GI, and SBI indices between primary and secondary Sjogren's were examined. The mean PI in pSS was 1.342 ±0.37, and in sSS was 1.314 ±0.495 (*p* = 0.829). The mean GI was 1.123 ±0.156 in pSS and 1.212 ±0.304 in sSS (*p* = 0.660). The mean SBI in pSS was 0.114 ±0.125, and in sSS was 0.186 ±0.292 (*p* = 0.980). Also, the mean USFR values were not statistically significant between the two groups of Sjogren's patients (*p* = 0.260). However, the mean DMFT in primary Sjogren's patients was 12.05 ±4.65, and in secondary patients, it was 9.31 ±4.15, which was statistically significant (*p* = 0.048) (Table 4).

Comparison of the quantitative indices in SS patients with regard to gender

The results showed no statistically significant difference in ADD-OHIP14, SC-OHIP14, age, GI, SBI, and DMFT in men and women with SS, but this difference was statistically significant for PI (*p* = 0.045). A comparison of the mean USFR in Sjogren's patients also showed that the mean in women was higher than in men (*p* = 0.026) (Table 5).

Table 3. Correlation relationships between the oral health-related quality of life (OHRQoL) dependent variables and the quantitative variables in both study groups

Quantitative variables	Case group				Control group			
	ADD-OHIP14		SC-OHIP14		ADD-OHIP14		SC-OHIP14	
	correlation coefficient	*p*-value	correlation coefficient	*p*-value	correlation coefficient	*p*-value	correlation coefficient	*p*-value
Age	0.024	0.876	0.141	0.356	−0.168	0.269	−0.105	0.491
PI	0.313*	0.036	0.354*	0.017	0.145	0.343	0.239	0.113
GI	−0.209	0.169	−0.179	0.240	0.070	0.648	0.108	0.479
SBI	−0.210	0.166	−0.190	0.211	0.105	0.492	0.138	0.365
DMFT	−0.019	0.899	−0.024	0.877	0.131	0.392	0.158	0.301
USFR	−0.90	0.559	−0.106	0.488	0.197	0.148	0.195	0.200
ADD_OHIP14	–	–	0.895**	0.000	–	–	0.958**	0.000
SC_OHIP14	0.895**	0.000	–	–	0.958**	0.000	–	–

* statistically significant ($p < 0.05$); ** statistically significant ($p < 0.015$).

Table 4. Comparison of the quantitative indices between the 2 groups of primary and secondary patients with Sjögren's syndrome (SS)

Quantitative variables	Primary SS			Secondary SS			*p*-value
	n	*M* ±*SD*	range	*n*	*M* ±*SD*	range	
ADD_OHIP14	19	21.00 ±10.30	3–39	26	18.50 ±13.93	0–50	0.493
SC_OHIP14	19	7.21 ±1.08	1–13	26	6.15 ±4.01	1–14	0.392
Age	19	47.68 ±8.42	32–63	26	52.77 ±6.73	37–70	0.037*
PI	19	1.342 ±0.370	0.5–2.0	26	1.314 ±0.495	0.2–2.0	0.829
GI	19	1.123 ±0.156	1.0–1.5	26	1.212 ±0.304	1.0–2.0	0.660
SBI	19	0.114 ±0.125	0–0.3	26	0.186 ±0.292	0–1.0	0.980
DMFT	19	12.05 ±4.65	2–21	26	9.31 ±4.15	3–18	0.048*
USFR	19	0.888 ±1.152	0.02–4.20	26	0.960 ±0.879	0.09–3.99	0.260

* statistically significant ($p < 0.05$).

Table 5. Comparison of the quantitative indices between the women and men with Sjögren's syndrome (SS)

Quantitative variables	Women			Men			p-value
	n	M ±SD	range	n	M ±SD	range	
ADD_OHIP14	40	18.75 ±11.58	0–50	5	26.0 ±18.47	7–50	0.224
SC_OHIP14	40	6.45 ±4.10	1–14	5	7.80 ±3.70	3–12	0.486
Age	40	50.25 ±7.28	32–64	5	53.60 ±11.97	37–70	0.373
PI	40	1.28 ±0.43	0.17–2.00	5	1.70 ±0.41	1.00–2.00	0.045*
GI	40	1.20 ±0.26	1–2	5	1.00 ±0.00	1	0.056
SBI	40	0.18 ±0.24	0–1	5	0.00 ±0.00	0	0.056
DMFT	40	10.53 ±4.60	2–21	5	10.00 ±4.36	5–17	0.811
USFR	40	1.01 ±1.02	0.01–4.20	5	0.30 ±0.18	0.10–0.59	0.026*

* statistically significant ($p < 0.05$).

Investigating the effect of the duration of the disease

Examination of the correlations between the studied quantitative variables and the duration of SS in Table 6 shows that there is a significant relationship between the studied indices ADD_OHIP14, SC_OHIP14, PI, GI, SBI, and USFR in patients with SS in relation to the duration of the disease. Still, there was a correlation with patient age ($r = 0.33$) which was also obvious ($p = 0.027$). Also, the mean DMFT in Sjogren's patients had a positive and significant relationship with the disease's duration (Table 6)

Table 6. Evaluation of correlation relations (Spearman' coefficient) between the duration of Sjögren's syndrome (SS) and the quantitative variables

Number	Quantitative variables	Duration of SS		
		n	correlation coefficient	p-value
1	ADD_OHIP14	45	–0.132	0.386
2	SC_OHIP14	45	–0.067	0.662
3	Age	45	0.329	0.027*
4	PI	45	0.036	0.814
5	GI	45	–0.147	0.336
6	SBI	45	–0.070	0.648
7	DMFT	45	0.297	0.048*
8	USFR	45	–0.057	0.708

* statistically significant ($p < 0.05$).

Effect of different drug therapies on the quantitative indices in SS patients

In this study, 100% of people used hydroxychloroquine and adjuvant drugs such as folic acid, calcium, and omega-3, 33% of patients were treated with methotrexate or prednisolone in addition to the above, and 11% of patients also used bromhexine. The results of a comparative analysis of the quantitative indices mean and the three types of prescribed drug treatments: (1) immunosuppressives with supplements and sialagogue; (2) immunosuppressives with supplements; and (3) immunosuppressive drugs with supplements and anti-rheumatic drugs using non-parametric multi-group comparisons with 95% accuracy showed that there was no significant differences or preferences between the mean of the variables ADD_OHIP14, SC_OHIP14, PI, GI, SBI, USFR, and DMFT (Table 7).

Table 7. Evaluation of the quantitative indicators with regard to the drugs used by Sjögren's syndrome (SS) patients (non-parametric comparative test – the Kruskal–Wallis method with 95% accuracy)

Quantitative variables	Drug groups used by the patients									p-value
	immunosuppressive + supplements + sialagogue			immunosuppressive + supplements			immunosuppressive + supplements + anti-rheumatic			
	n	M ±SD	range	n	M ±SD	range	n	M ±SD	range	
ADD_OHIP14	5	21.40 ±9.40	9–34	25	20.36 ±11.03	3–41	15	17.6 ±15.74	0–50	0.464
SC_OHIP14	5	7.00 ±3.94	2–13	25	5.87 ±4.17	1–14	15	6.60 ±4.02	1–12	0.588
PI	5	1.33 ±0.20	1.17–1.67	25	1.39 ±0.45	0.17–2.00	15	1.21 ±0.49	0.17–1.83	0.284
GI	5	1.03 ±0.07	1.00–1.17	25	1.21 ±0.27	1.00–2.00	15	1.16 ±0.25	1.00–1.83	0.256
SBI	5	0.07 ±0.09	0.00–17.00	25	0.19 ±0.26	0.00–1.00	15	0.13 ±0.23	0.00–0.83	0.587
DMFT	5	13.00 ±5.83	7.00–21.00	25	9.24 ±4.31	2.00–17.00	15	11.67 ±4.05	4.00–20.00	0.198
USFR	5	0.44 ±0.61	0.01–1.4	25	0.89 ±0.99	0.02–4.00	15	1.16 ±1.07	0.30–4.20	0.108

Discussion

The present study compared OHRQoL in patients with primary and secondary SS and healthy individuals. Based on the study results, PI, GI, SBI, and DMFT indices did not show a statistically significant difference between the two groups. USFR was significantly different between patients with SS and healthy individuals ($p = 0.002$).

The results of many studies have linked SS to poor periodontal status for more than three decades,[28,29] with PI, GI, and bleeding on probing (BOP) indices higher in Sjogren's patients than in healthy patients. In a meta-analysis of 512 studies examining PI, GI, probing pocket depth (PPD), clinical attachment loss (CAL),[30] and DMFT in patients with SS, only 10 studies were eligible for the meta-analysis. Four studies showed a statistically significant difference in the GI between patients and healthy individuals,[12,31–33] and 5 studies reported PLI in Sjogren's patients higher than controls.[32–36] The final results of the meta-analysis between 163 patients with SS and 164 healthy individuals did not show a statistically significant difference in the PI and GI in these two groups.[37] Some studies have reported a higher risk of periodontal involvement in patients with SS than in healthy individuals.[28,31] In contrast, other studies have not reported an increase in periodontal involvement in Sjogren's patients.[32,33,35,36]

Finally, the meta-analysis results in 2019 showed that despite the higher rates of periodontal disease in Sjogren's patients compared to healthy individuals, the statistical difference between the two groups was not significant.[37]

In another review study on the relationship between SS and a patient's periodontal status, 17 studies were reviewed, with only 8 studies being included in the meta-analysis.[33] The PI and GI were higher than in healthy individuals in 4 studies of Sjogren's patients, and the BOP in the case group was higher than in the control group.[38] A meta-analysis of 303 patients with primary and secondary SS with 288 healthy individuals showed that the GI was statistically significant in primary and secondary SS compared to the control group. The meta-analysis reported differences in the results related to PI, GI, BOP, and PPD due to differences in the examiners used in the different studies. This means that these indicators are subjective, and each examiner may use different pressures to examine these indicators. Even one examiner may not have the same examination pressure on periodontal tissues during the study.[38] Good oral hygiene and less severe disease are cited in a study to explain the lack of significant association between SS and periodontal disease.[39]

Also, there was no significant relationship between periodontal involvement in Sjogren's patients and healthy individuals due to long-term use of drugs such as NSAIDs, DMARDs, and immunosuppressive drugs, which can effectively treat periodontal inflammatory responses.[40]

In our study, the lack of a significant relationship between GI, PI, and SBI indices between the case and control groups and the reasons mentioned in the above examinations can also be related to the inclusion or exclusion criteria of the participants. Destructive and influential factors on the periodontium, especially smoking, and diabetes were among the participants' exclusion criteria in our study. To accurately evaluate the effect of the disease on the periodontal status, we applied strict criteria in selecting individuals for the two groups, which some studies neglected.[12,41]

In this study, the DMFT index did not show a statistically significant relationship between the two groups. In Antoniazzi's study, a tooth missing in patients with pSS was not statistically significant compared to the healthy control group.[31] The results of a meta-analysis mentioned DMFT in the group of Sjogren's patients to be higher than in the control group. It should be noted that one of the possible reasons for this result in comparison with our study is the role of smoking and its use which was reported in 7 studies of this meta-analysis.[12,31,33,35–37,42] Despite the lack of a significant relationship between the DMFT index between case and control groups in our study, there was a statistically significant difference in DMFT between primary and secondary SS compared to patients with primary and secondary SS ($p = 0.048$).

Kalk noted that the submandibular and sublingual salivary flow rate in SS was lower than usual.[43] This result was consistent with our study and some studies that reported higher DMFT in patients with pSS due to decreased salivary gland function and decreased salivary buffering capacity compared to sSS.[12,39,41] Hyposalivation can also be another cause of higher DMFT in pSS. In our study, 42% of people with pSS had hyposalivation, while 34% of people with sSS showed decreased salivary gland function.

The study of oral health variables related to the quality of life in Sjogren's patients with healthy individuals showed that ADD-OHIP-14 ($p = 0.037$) and SC-OHIP-14 ($p = 0.025$) were statistically significantly different between the two groups. Participants in all 7 subgroups and 14 questions showed a statistically significant difference. The effects of SS on an individual's quality of life have been evaluated in various studies.[19,44,45] In these articles, the patient's quality of life with SS was lower than that of healthy individuals in the community, especially regarding severe limitations in the physical functioning of SS patients.[39,46]

Rusthen examined the effects of salivation, halitosis, chemosensory function, burning sensation of the tongue (BST), and OHRQoL in people with pSS and healthy individuals. The results showed that the case group had a lower mean OHRQOL in the following 4 subgroups of physical limitation, functional limitation, psychological limitation, and social limitation than in the control group. Stimulated and unstimulated salivary flow rates were significantly lower in Sjogren's patients with a higher prevalence of dysgeusia, BST, and halitosis in

SS, which is a sign of salivary and chemosensory dysfunction.[25] Enger evaluated systemic health effects on the OHRQoL in patients with pSS and a healthy group. In this study, higher oral distress in Sjogren's patients led to decreased OHRQoL, which had significant effects on health-related quality of life. Patients with SS in this study had a significant difference from the control group in all 14 questions. The biggest differences were seen in the subgroups of physical pain, psychological distress, and functional limitation.

Enger noted that xerostomia plays a crucial role in causing oral complications, increasing tooth decay, and leading to frequent dental visits in Sjogren's patients. It was also mentioned in this study that oral distress has significant effects on a patient's physical and mental health, and the powerful impact on their self-esteem, self-confidence, and social relationships is undeniable. Therefore, the main focus on oral health aspects in patients with SS is strongly recommended to improve their systemic health.[12]

This study is the first study to evaluate the effect of periodontal indices, DMFT, and USFR on OHRQoL in patients with SS. Despite the limited number of patients, especially patients with pSS, we used strict inclusion criteria to evaluate the effect of the disease on oral health care. We suggest that more multicenter studies be conducted with a larger sample size over a more extended period of time in the future. Another limitation of the present study was that periodontal variables such as the CAL and PPD were not measured in these patients because normal periodontal conditions or mild gingivitis were observed in the small sample size of the patients with SS we examined. It is suggested that these periodontal factors be examined in future studies.

Conclusions

In our study to evaluate OHRQoL as a dependent variable concerning the quantitative variables studied, including PI, GI, SBI, DMFT, and USFR, only the PLI in the case group was statistically significant and had a positive correlation with OHRQoL. According to the results, in addition to regular examinations and the drug treatment of patients with SS, regular oral examinations are recommended to check for dry mouth and prevent its consequences, such as dental caries and periodontal disease.

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee at Mazandaran University of Medical Sciences, Sari, Iran (IR.MAZUMS.REC.1398.6148). Written informed consent was obtained from all participants prior to the commencement of the study.

Data availability

All data generated and/or analyzed during this study is included in this published article.

Consent for publication

Not applicable.

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Original paper

Oral health-related quality of life in a group of patients with rheumatoid arthritis

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Conflict of interest
None declared

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Abstract

Background. Rheumatoid arthritis (RA) and periodontitis (PD) are chronic diseases that are associated with connective tissue and bone destruction, which affects the quality of life of the people suffering from these conditions. The identification of social conditions and the determinants of RA and PD would permit the elaboration of policies and strategies based on social reality.

Objectives. The aim of the present study was to identify the relationship between oral health-related quality of life (OHRQoL) and the indicators of general health and oral health in patients with RA.

Material and methods. A cross-sectional study involving 59 patients with RA was conducted between 2019 and 2020. Demographic, general health, periodontal, and oral health parameters were collected. In addition, the Oral Health Impact Profile-14 (OHIP-14) questionnaire was administered to each patient. A description of the OHIP-14 dimensions according to different variables was performed. The relationship between OHRQoL and general/oral health indicators was analyzed with logistic and linear regression analyses.

Results. The highest OHIP-14 scores were found in people that were 60 years of age and over, single, had low educational achievements, a low socioeconomic status, were unemployed, and had no health affiliation. In the adjusted model, the prevalence of the impact on OHRQoL was 1.34 (1.10–5.29) times greater in those with erosive RA than in those without, and 2.22 (1.16–29.50) times greater in those who self-reported morning stiffness. Regarding the stage of PD, those with stage IV had a prevalence of the impact on the OHRQoL of 70%, an average extent of 3.4 ±4.5 and a severity score of 11.5 ±22.0, with statistically significant differences.

Conclusions. The dimensions with the greatest impact on the OHRQoL of patients were physical pain, discomfort and psychological disability. The type of RA and the severity of PD are indicators of worse scores on the OHRQoL scale.

Keywords: periodontitis, quality of life, oral health, rheumatoid arthritis

Introduction

Chronic diseases are characterized by a long duration and slow progression. They manifest themselves during adulthood and carry with them a number of associated consequences. Most of the time, the pain, anxiety, and disability caused by these chronic diseases compromise the health-related quality of life (HRQOL) of patients.[1] Both rheumatoid arthritis (RA) and periodontitis (PD) are chronic diseases that cause connective tissue and bone destruction.[2] These two diseases are currently considered to share etiopathological mechanisms and therefore are said to be related both biologically and epidemiologically.[2] RA is defined as an inflammatory, systemic, autoimmune disease involving the loss of control of the autologous immune response leading to effector elements (humoral and cellular) that will generate a specific immune response against the patient's own autoantigens or tissues.[3,4] Currently, the etiology of RA is not entirely clear, although the interrelationship between genetic and environmental factors plays an important role in a patient's susceptibility to RA.[4] Its prevalence among the world population varies between 0.2% and 5.0% and has a prevalence of approximately 1% in Colombia. It occurs more often in women than in men, with a ratio of 4:1, and increases with age.[5]

Oral health is closely related to health in general and the HRQOL perceived by people. Functional, social, and psychological aspects are essential for the integral development of the human being throughout life, which in turn allows optimal interpersonal relationships.[6] The presence of PD can cause additional complications such as pain, discomfort, and low self-esteem.[7] According to various studies, patients suffering from rheumatic diseases have a reduced quality of life, especially those presenting with oral manifestations and physical effects due to oral and dental diseases which cause psychosocial deficiencies.[8]

Oral health is an important component of one's HRQOL, and different approaches have been made to isolate these effects through the preparation of different questionnaires, among which is the Oral Health Impact Profile (OHIP) by Locker. It is used to conceptualize a biopsychosocial approach to the deficiencies, disabilities, and handicaps of oral health.[6] The most recent version of this instrument is the OHIP-14, which evaluates the impact of oral diseases on HRQOL using seven factors: 1) functional limitation: problems pronouncing words, worsening sense of taste; 2) physical pain: mouth pain, discomfort/pain with eating; 3) psychological discomfort: teeth self-awareness, feelings about the teeth or mouth appearance; 4) physical disability: problems with diet/eating; 5) psychological disability: problems relaxing, sleeping, or feeling embarrassed because of teeth/mouth problems; 6) social disability: difficulties in social relationships, or in carrying out daily activities; and 7) handicap: the feeling of a less satisfying life, inability to function properly due to dental problems.[6,9] The hierarchy captures outcomes that have an increasingly disruptive impact on people's lives. The instruments used for analyzing the OHIP have been validated in several countries.[6,10–13] On the other hand, there is an instrument that measures the oral health self-perception in patients with the periodontal disease without leaving aside the multidimensional theoretical model of oral health.[14]

Although the health and oral health-related quality of life (OHRQOL) has been studied in populations with different characteristics and RA patients.[15–17] However, there are no local (Colombia and Medellín) studies using OHRQOL instruments to investigate the quality of life of RA patients currently available. Given the scarcity of research on the aforementioned aspect, this study is needed to provide information that encourages the creation of a research line that promotes prevention strategies in patients with chronic diseases by advocating for an interdisciplinary approach.

Considering the above, this study aimed to determine the relationship between OHRQOL and general and oral health indicators in a group of patients with RA, as well as its associated sociodemographic and clinical factors.

Methods

Design and setting

A cross-sectional study was carried out using a structured survey, a periodontal exam, and a hematological exam in adult patients older than 18 years, who consulted a clinic specializing in the treatment of patients with RA in Medellín, Colombia. This manuscript was written according to the STROBE guidelines for observational studies.[18]

Participants and selection criteria

RA patients were recruited between March 2019 and March 2020 from a specialized clinic in Medellín, Colombia. Individuals were included according to the following criteria: age ≥18 years, RA diagnosis according to the American College of Rheumatology[19] with a disease activity score in 28 joints – C-reactive protein (DAS28-CRP) value ≥3.2 – and no changes in RA medications in the previous 3 months and throughout follow-up, and at least 15 teeth excluding third molars. People who reported periodontal treatment or antibiotic use in the previous 3 months, HIV, liver disease, head and neck radiation therapy, pregnancy, and cyclosporine use were excluded. Smoking, hypertension medication use, and hyperlipidemia were not exclusion criteria and were recorded accordingly. In the consultation with the treating physician, the selection criteria were confirmed. For example, patients on cyclosporine for a transplant were not included in the study, while hypertensive patients controlled with medication were included since the results would not be affected. For patients who met these criteria, we explained the purpose of the research. Those who agreed to participate were provided informed consent forms and an explanation of the procedure, and all their questions were answered. Signed informed consent forms were collected prior to the initiation of the research study.

Information gathering

A survey containing variables such as sociodemographic and clinical factors, RA self-perception, and questions from the OHIP-14 was administered. Subsequently, appointments were scheduled for blood tests to be performed at the Universidad de Antioquia and a periodontal examination for non-surgical treatment or prophylaxis, as well as an explanation about oral hygiene.

OHIP-14 questionnaire

Regarding the OHRQOL, the results were obtained through the use of the OHIP-14, which consisted of 14 questions distributed in 7 dimensions: functional limitation; physical pain; psychological discomfort; physical disability; psychological disability; social disability; and handicap. Each question has 5 answer options, and each one is assigned a score: 0 – never; 1 – almost never; 2 – sometimes; 3 – frequently; and 4 – always.[6,9,10]

The OHIP-14 analysis allowed the use of 3 summary variables of the functional and psychological consequences of oral health problems[20]:

1) Prevalence: percentage of individuals who report one or more items "frequently" or "always";
2) Extension: number of items reported as "frequently" or "always"; and
3) Severity: the sum of all the ordinal value responses, and scores in a range from 0 to 56, with the higher value indicating a greater impact of oral health on one's quality of life.

Such measures have been used in other research on the international level[20,21] and in the city of Medellín.[22,23]

Physical examination

Patients received a full mouth periodontal examination, excluding the third molars. The following clinical parameters were recorded with a millimeter periodontal probe (UNC15) at 6 sites: probing pocket depth (PPD); bleeding on probing (BOP); and clinical attachment loss (CAL). The stage of PD was determined according to the current classification of periodontal disease.[24] At the same time, we identified cases of periodontal health and gingival diseases and conditions on an intact and a reduced periodontium.[25] The standardization was carried out by a periodontist dentist in patients with characteristics similar to those of our study. They underwent dental physical examination and measurements of the oral clinical variables until a kappa, and a 0.80 or higher intraclass correlation coefficient (ICC) was obtained.

Serological markers: all patients underwent peripheral blood tests for anti-citrullinated protein antibodies (ACPAs; U/mL), ultrasensitive C-reactive protein (CRP; mg/L), and rheumatoid factor (RF; U/mL). Serological tests were performed in a clinical laboratory.

RA patients were examined by a trained rheumatologist during the study and the rheumatologist confirmed the diagnosis. Medical information was obtained from their medical records, including RA duration and current medication for RA (non-steroidal anti-inflammatory drugs (NSAIDs), biological and non-biological disease-modifying antirheumatic drugs (DMARDs), and corticosteroids). Non-biologic DMARDs included hydroxychloroquine, methotrexate, sulfasalazine, and leflunomide. Biologic DMARDs included adalimumab, etanercept, abatacept, golimumab, infliximab, rituximab, and tocilizumab.

Statistical analysis

An initial exploratory and descriptive analysis was performed to determine the distribution of the variables, and appropriate tests (Kolmogorov–Smirnov) were performed to assess normality. Continuous variables are presented with the corresponding central tendency. Dispersion measures and parametric or non-parametric tests were used to determine the differences between the indicated groups. Categorical variables are presented as frequencies, and differences between the groups were tested using the χ^2 tests.

In addition, to determine the influence of the independent variables on OHIP and its dimensions, Spearman's rank correlation coefficient and a multivariate linear regression analysis were performed to evaluate the simultaneous and reciprocal effect of the explanatory variables. Compliance with the assumptions of linearity, non-collinearity, normality, constant variance, and correlation of residuals was determined.

A logistic regression analysis was carried out to test the association between the OHIP covariates and prevalence. The crude odds ratio (*OR*, 95% confidence interval (*CI*)) and the adjusted *OR* of the covariates are presented in the multivariate analysis, in which variables were included according to the Hosmer–Lemeshow criterion ($p \leq 0.25$) or according to biological plausibility. The power of the study was calculated at 87.3% for cross-sectional studies. All data was analyzed using IBM SPSS Statistics for Windows, v. 25.0 (IBM Corp., Armonk, USA), and statistical significance was assumed when the p-value was ≤0.05.

Results

Out of the 59 RA patients, 81.4% were women. Considering both sexes, individuals were aged between 30 and 73 years (average 54.5 ±9.2 years) and had a disease duration between 1 and 45 years (average 10.2 ±8.2 years). Additionally, 72.9% of the patients at the time of the study did not work. The dental variables measured for all patients were 23.1 ±3.9 teeth, CAL average of 3.2 ±2.2 mm, PPD average of 3.1 ±1.9 mm, and BOP average of 20.5 ±14.3%. The summary of the blood parameters for the study population was as follows: ACPAs of 222.7 ±298.6 U/mL, RF of 218.1 ±505.5 U/mL, and CRP of 6.9 ±10.1 mg/L.

OHIP-14 results

The results of the OHIP-14 included comparisons regarding the prevalence, extent, and severity, according to the sociodemographic variables, of RA and periodontal diseases, as well as the analysis of the dimensions.

The prevalence of the impact on OHRQOL is shown in Table 1. The highest impact was found among women (83.3%), married people (61.1%), the middle socioeconomic class (66.7%), people affiliated with the health care contributory and special regime (61.1%), and people who did not work (77.8%). Similar results were observed for the OHIP extent, with a higher average (2.69 ±3.16) in people aged 60 years and over. Regarding severity, higher median scores were observed in people with no education (9.0 (19.0)), lower class (8.0 (21.0)), and those who were not affiliated with the health care system (13.0 (–)).

When comparing the impact of OHIP according to habits, systemic conditions, and periodontal conditions, a higher prevalence was observed in those who did not smoke, did not consume alcohol, did not exercise, and consumed corticosteroids and DMARDs. The average extent was higher in those who did not exercise (2.3 ±3.7), had hypertension (2.5 ±3.9), had osteoporosis (2.7 ±4.7), used corticosteroids (2.1 ±3.5), and was on DMARDs (1.9 ±3.3). Regarding severity, the highest scores were reported in patients with diabetes (9.0 (19.0)). In addition, statistically significant differences in terms of prevalence, extent, and severity were found regarding the stage of PD. Those with stage IV occurred at a prevalence of 70.0%, an extension of 3.4 ±4.5, and a severity of 11.5 (22.0) (Table 2).

Table 3 shows statistically significant differences in RA type according to the number of affected joints and OHIP prevalence and extent, with a prevalence of 77.8% for polyarticular arthritis and an average extent of 5.0 ±4.4 for those with oligoarticular RA and severity with a median value of 21.0 (30.0). Regarding RA symptoms self-perception, those who reported having swelling, pain, and morning stiffness had a higher OHIP prevalence (72.2%). In terms of extension, a higher difference was found in those who reported pain (2.2 ±3.6). On the topic of severity, there was a higher difference between those who reported morning stiffness (7.0 (19)) compared to those who did not. In those who reported

Table 1. Summary outcomes of the Oral Health Impact Profile-14 (OHIP-14) questionnaire as a proxy of oral health-related quality of life (OHRQoL) according to sociodemographic variables in patients with rheumatoid arthritis (RA) (*N* = 59)

Variables		Sample		OHRQoL (summary outcomes of OHIP-14)			
		n	%	prevalence (%)	extent (*M* ±*SD*)	severity (OHIP-14 score)	
						M ±*SD*	*Me* (*IQR*)
Sex	male	11	18.6	16.7	1.6 ±2.6	9.7 ±10.6	8.0 (20.0)
	female	48	81.4	83.3	1.8 ±3.4	10.1 ±11.5	6.0 (15.0)
Age [years]	27–59	42	71.2	55.6	1.4 ±3.2	9.0 ±11.1	6.0 (11.0)
	60 and more	17	28.8	44.4	2.7 ±3.2	12.5 ±11.8	8.0 (22.0)
Marital status	married	35	59.3	61.1	1.5 ±2.5	9.2 ±9.8	7.0 (17.0)
	living alone	24	40.7	38.9	2.2 ±3.9	11.1 ±13.2	5.0 (18.0)
Education	≤primary	22	37.3	44.4	2.4 ±3.8	12.5 ±12.5	9.0 (19.0)
	secondary	31	52.5	44.4	1.4 ±2.8	8.7 ±10.7	4.0 (14.0)
	≥university	6	10.2	11.2	1.2 ±2.0	7.2 ±8.1	4.5 (12.0)
Socioeconomic status	low	13	22.0	33.3	2.8 ±4.2	12.6 ±14.1	8.0 (21.0)
	medium	42	71.2	66.7	1.6 ±3.0	9.4 ±10.8	5.0 (18.0)
	high	4	6.8	0.0		8.2 ±4.1	7.5 (8.0)
Social security (health insurance)	none	2	3.4	5.6	2.0 ±2.8	13.0 ±12.7	13.0
	paying regime/special	43	72.9	61.1	1.6 ±3.0	9.6 ±10.4	7.0 (16.0)
	subsidized	14	23.7	33.3	2.2±4.0	10.7 ±14.0	6.0 (16.0)
Employment situation	employed	16	27.1	22.2	1.1 ±2.4	7.2 ±9.0	5.0 (10.0)
	unemployed	43	72.9	77.8	2.0 ±3.4	11.0 ±11.9	7.0 (19.0)

M – mean; *SD* – standard deviation; *Me* – median; *IQR* – interquartile range; statistical significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (non-parametric tests: Mann–Whitney *U* test for dichotomous variables; Kruskal–Wallis test for polychotomous variables; and χ^2 test for categorical variables).

Table 2. Summary outcomes of the OHIP-14 questionnaire as a proxy of OHRQoL according to habits, and periodontal and systemic condition in patients with rheumatoid arthritis (RA) ($N = 59$)

Variables		Sample		OHRQoL (summary outcomes of OHIP-14)			
		n	%	prevalence (%)	extent (*M* ±*SD*)	severity (OHIP-14 score)	
						M ±*SD*	*Me* (*IQR*)
Smoking	yes	4	6.8	11.1	1.2 ±1.9	9.5 ±9.2	8.0 (18.0)
	no	55	93.2	88.9	1.8 ±3.3	10.0 ±11.5	6.0 (16.0)
Alcohol consumption	yes	3	5.1	5.6	0.33 ±0.6	7.3 ±1.1	8.0 (–)
	no	56	94.9	94.4	1.8 ±3.3	10.1 ±11.6	6.0 (18.0)
Practicing exercise (sports)	yes	22	62.7	27.8	1.1 ±2.2	9.3 ±9.6	7.5 (13.0)
	no	37	37.3	72.2	2.3 ±3.7	10.9 ±12.4	6.0 (20.0)
Diabetes	yes	6	10.2	11.1	1.8 ±2.8	10.2 ±8.8	9.0 (19.0)
	no	53	89.8	88.9	1.7 ±3.2	10.0 ±11.6	6.0 (15.0)
Arterial hypertension	yes	19	32.2	38.9	2.5 ±3.9	11.7 ±13.9	8.0 (21.0)
	no	40	67.8	61.1	1.5 ±2.9	9.5 ±10.2	6.0 (13.0)
Osteoporosis	yes	10	16.9	16.7	2.7 ±4.7	11.9 ±15.6	4.5 (23.0)
	no	49	83.1	83.3	1.6 ±2.9	9.6 ±10.5	6.5 (15.0)
NSAIDs consumption	yes	8	13.8	11.1	1.0 ±2.1	9.2 ±7.8	8.0 (13.0)
	no	50	86.2	88.9	1.9 ±3.4	10.4 ±11.9	6.0 (19.0)
Corticosteroids consumption	yes	35	60.3	61.1	2.1 ±3.5	11.0 ±12.2	7.0 (20.0)
	no	23	39.7	38.9	1.4 ±2.7	8.5 ±10.1	6.0 (11.0)
DMARDs consumption	yes	53	91.4	94.4	1.9 ±3.3	10.5 ±11.8	6.0 (19.0)
	no	5	8.6	5.6	1.2 ±2.6	8.8 ±7.6	8.0 (11.0)
Periodontitis	yes	31	59.6	32.3	1.9 ±3.5	10.5 ±12.0	8.0 (14.0)
	no	21	40.4	37.5	1.6 ±2.7	9.8 ±10.5	4.0 (19.0)
Stage of periodontal disease†	I	0	0.0	–	–	–	–
	II	1	3.2	0.0*	–	–	–
	III	18	58.1	30.0*	0.9 ±2.4*	6.9 ±8.9*	5.0 (11.0)*
	IV	12	38.7	70.0*	3.4 ±4.5*	16.6 ±14.2*	11.5 (22.0)*

NSAIDs – non-steroidal anti-inflammatory drugs; DMARDs – disease-modifying antirheumatic drugs; statistical significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (non-parametric tests: Mann–Whitney *U* test for dichotomous variables; Kruskal–Wallis test for polychotomous variables; and χ^2 test for categorical variables). † Classification of periodontitis based on the stages defined by severity (according to the level of interdental clinical attachment loss (CAL), radiographic bone loss and tooth loss), complexity, extent, and distribution.[24]

fatigue and depression, the severity (9.0 (19)) was not statistically significantly different.

When comparing the OHIP-14 dimensions in patients who have and do not have PD, higher indicators were found in those who experienced it in the dimensions of physical pain (1.5 (4.0)) and psychological disability (1.5 (3.0)) (Table 4).

Table 5 shows the correlations between the OHIP-14 dimensions and the sociodemographic, habits, clinical dental variables, and RA-related blood parameters (all variables of quantitative nature). A statistically significant correlation between age and the dimensions of physical (0.35) and psychological (0.27) disability was observed. Also, a statistically significant correlation was found between the number of teeth present and all dimensions, the extension, and OHIP-14 total impact. The average BOP was correlated with the dimensions of physical (0.30), psychological (0.46), and social (0.27) disability, as well as with handicap (0.43). RA blood parameters showed low correlations with the different dimensions. It is observed that ACPAs presented the highest inverse correlation with functional limitation, while RF presented a positive correlation with the dimension of physical pain. These correlations were not statistically significant.

In the linear regression model, age was a positive predictive value (0.38) for the physical disability dimension, which is in contrast to the duration in years of RA, which was a negative predictive value (−0.30). The number of teeth present was a negative predictive value for the OHIP-14 total score, psychosocial disability dimension, and handicap dimension (−0.34, −0.36 and −0.31, respectively), meaning the fewer the number of teeth, the worse the reported indicators. The average BOP had a positive predictive value for the psychological disability dimension (0.31). These results were explained by the model between 10% and 30% (Table 6).

Table 3. Summary outcomes of the OHIP-14 questionnaire as a proxy of OHRQoL according to clinical and self-perception variables in patients with rheumatoid arthritis (RA) (*N* = 59)

Variables		Sample		OHRQoL (summary outcomes of OHIP-14)			
		n	%	prevalence (%)	extent (*M* ±*SD*)	severity (OHIP-14 score) *M* ±*SD*	severity (OHIP-14 score) *Me* (*IQR*)
RA-related clinical variables							
Type of RA	polyarticular	53	89.8	77.8*	1.5 ±3.0*	9.3 ±10.8	6.0 (13.0)
	oligoartícular	5	8.5	22.2*	5.0 ±4.4*	18.4 ±15.1	21.0 (30.0)
	monoarticular	1	1.7	0.0*			
RA activity	remission	30	53.6	55.6	1.5 ±2.6	9.0 ±9.2	6.5 (13.0)
	low	6	10.7	11.1	2.6 ±3.4	13.0 ±10.9	9.0 (18.0)
	moderate	10	17.9	16.7	2.1 ±3.7	10.2 ±13.9	2.0 (23.0)
	high	10	17.8	16.7	2.6 ±4.6	13.0 ±15.6	4.0 (21.0)
Erosive RA	yes	22	42.3	50.0	1.6 ±2.7	9.6 ±9.8	7.0 (20.0)
	no	30	57.7	50.0	1.2 ±2.6	8.2 ±9.6	5.0 (14.0)
Alternative therapies	yes	1	1.7	5.9			
	no	57	98.3	94.1	1.7 ±3.2	9.8 ±11.4	6.0 (15.0)
Self-perception variables							
Swelling	yes	44	74.6	72.2	1.9 ±3.3	10.8 ±11.9	7.0 (19.0)
	no	15	25.4	27.8	1.4 ±2.9	7.7 ±9.3	6.0 (7.0)
Pain	yes	46	78.0	72.2	2.2 ±3.6	10.8 ±12.0	7.0 (21.0)
	no	13	22.0	27.8	1.6 ±3.1	9.8 ±11.2	6.0 (14.0)
Morning stiffness	yes	16	27.1	72.2	1.8 ±3.2	10.6 ±11.5	7.0 (19.0)
	no	43	72.9	27.8	1.6 ±3.1	8.6 ±11.0	4.5 (10.0)
Fatigue and depression	yes	27	45.8	55.6	1.9 ±3.2	11.3 ±10.8	9.0 (19.0)
	no	32	54.2	44.4	1.6 ±3.2	8.4 ±11.8	4.0 (8.0)
Family support	yes	59	100.0	100.0	1.0 ±2.1	8.5 ±9.5	5.5 (14.0)
	no	0	0.0	0.0	–	–	–

Statistical significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (non-parametric tests: Mann–Whitney *U* test for dichotomous variables; Kruskal–Wallis test for polychotomous variables; and χ^2 test for categorical variables).

Finally, the logistic regression analysis aiming to observe associations between the OHIP-14 prevalence indicator and the variables of person, habits, and RA clinics in patients with and without PD was performed (Table 7). The results showed that after adjusting for sociodemographic variables, the prevalence of the impact on HRQOL was 1.3 (95% *CI*:1.10–5.29) times more in patients with erosive RA than in those without it, and 2.2 (95% *CI*:1.16–29.50) times more in those who self-reported morning stiffness. On the other hand, in the crude model, an association was observed between the prevalence of polyarticular RA and having a periodontal stage between III and IV. Finally, having access to an alternative treatment for RA was regarded as a protective factor.

Table 4. OHIP-14 dimensions according to the presence of periodontitis in patients with rheumatoid arthritis (RA) (*N* = 59)

OHIP-14 dimensions	All		Periodontitis		No periodontitis		*p*-value
	M ±*SD*	*Me* (*IQR*)	*M* ±*SD*	*Me* (*IQR*)	*M* ±*SD*	*Me* (*IQR*)	
Functional limitation	0.9 ±1.8	0.0 (1.0)	0.9 ±2.1	0.0 (0.0)	0.9 ±1.3	0.0 (2.0)	0.432
Physical pain	2.3 ±2.4	2.0 (4.0)	2.4 ±2.7	1.5 (4.0)	1.9 ±2.1	1.0 (4.0)	0.797
Psychological discomfort	2.2 ±2.4	2.0 (4.0)	1.9 ±2.4	0.5 (4.0)	2.5 ±2.2	2.0 (4.0)	0.154
Physical disability	1.7 ±2.3	0.0 (3.0)	1.8 ±2.3	0.0 (4.0)	1.6 ±2.5	0.0 (3.0)	0.650
Psychological disability	1.6 ±2.0	1.0 (3.0)	1.8 ±2.1	1.5 (3.0)	1.4 ±1.9	0.0 (3.0)	0.563
Social disability	0.5 ±1.2	0.0 (0.0)	0.5 ±1.4	0.0 (0.0)	0.4 ±0.9	0.0 (1.0)	0.891
Handicap	0.9 ±1.7	0.0 (2.0)	0.8 ±1.6	0.0 (2.0)	0.9 ±1.6	0.0 (2.0)	0.745

Mann–Whitney/Kruskal–Wallis tests.

Table 5. Correlation between the OHIP-14 dimensions and different variables in patients with rheumatoid arthritis (RA) ($N = 59$)

Variables	Extent	OHIP-14 score	Functional limitation	Physical pain	Psychological discomfort	Physical disability	Psychological disability	Social disability	Handicap
Age	0.20	0.25	0.18	0.14	0.25	0.35**	0.27*	0.13	0.05
RA duration	−0.14	0.08	−0.10	0.11	0.04	0.00	0.06	0.00	−0.06
Number of sleeping hours	−0.06	−0.06	−0.25	0.01	−0.10	−0.05	−0.07	−0.24	−0.03
Number of teeth	−0.42**	−0.44**	−0.28*	0.28*	−0.36**	−0.40**	−0.51**	−0.31*	−0.041**
Mean BOP	0.33*	0.33*	0.10	0.25	0.17	0.30*	0.46**	0.27*	0.43**
Mean CAL	0.08	0.01	−0.11	−0.02	−0.05	0.07	0.18	−0.06	−0.00
Mean PPD	0.09	0.11	0.02	0.10	−0.06	0.03	0.23	0.21	0.19
ACPAs	−0.18	−0.06	−0.30	−0.05	−0.05	−0.01	−0.03	0.04	−0.02
RF	−0.07	0.06	−0.03	0.11	0.06	0.04	0.07	−0.01	0.06
CRP	−0.02	0.01	−0.05	0.07	0.02	−0.03	0.05	−0.02	−0.09

BOP – bleeding on probing; PPD – probing pocket depth; CAL – clinical attachment loss; ACPAs – anti-citrullinated protein antibodies; RF –rheumatoid factor; CRP – C-reactive protein; * statistically significant correlation at $p < 0.05$ (bilateral); ** statistically significant correlation at $p < 0.01$ (bilateral).

Table 6. Multivariate lineal regression model for the scores in the OHIP-14 dimensions in patients with rheumatoid arthritis (RA) ($N = 59$)

OHIP-14 dimensions (dependent variables)	Independent variables included in the model	Standardized coefficient (β)	Determination coefficient (%)
Extent	age	0.15	17.0
	RA duration	−0.05	
	number of teeth	−0.32	
	mean BOP	0.20	
	ACPAs	−0.14	
OHIP-14 score	age	0.10	17.0
	RA duration	−0.34*	
	number of teeth	0.15	
	mean BOP	−0.02	
Functional limitation	age	0.07	5.0
	RA duration	−0.06	
	number of sleeping hours	−0.21	
	number of teeth	−0.22	
	mean BOP	0.22	
	mean CAL	0.043	
	ACPAs	−0.02	
Physical pain	age	0,21	4.3
	RA duration	−0,22	
	number of sleeping hours	−0,09	
	number of teeth	−0,11	
	mean BOP	0,12	
	mean PPD	0,10	
	RF	0,17	
Psychological discomfort	age	0.22	8.0
	RA duration	−0.10	
	number of sleeping hours	−0.50	
	number of teeth	−0.31	
	mean BOP	0.06	
	ACPAs	−0.03	
	RF	−0.10	
Physical disability	age	0.38*	24.4
	RA duration	−0.30*	
	number of teeth	−0.25	
	mean BOP	0.11	
	ACPAs	−0.56	
	RF	0.13	
Psychological disability	age	0.10	29.2
	RA duration	−0.09	
	number of teeth	−0.36*	
	mean BOP	0.31*	
	ACPAs	−0.04	
	RF	0.06	
Social disability	age	0.08	6.8
	RA duration	0.03	
	number of sleeping hours	−0.08	
	number of teeth	−0.08	
	mean BOP	0.09	
	RF	0.08	
Handicap	age	0.13	10.9
	number of teeth	−0.31*	
	mean BOP	0.19	
	mean PPD	0.12	
	ACPAs	−0.08	
	RF	−0.03	
	CPR	−0.07	

Statistical significance: * $p < 0.05$; ** $p < 0.01$; *** $p <0.001$.

Table 7. Evaluating the association between the prevalence outcome of OHIP-14 and different variables by means of multivariate regression logistic analysis in patients with rheumatoid arthritis (RA) (*N* = 59)

Variable		Prevalence outcome of OHIP-14	
		crude *OR* (95% *CI*)	adjusted *OR*† (95% *CI*)
Sex	male	1.00	1.00
	female	1.21 (0.28–5.22)	1.45 (0.30–6.87)
Age [years]	27–59	1.00	1.00
	60 and more	2.80 (0.87–9.33)	2.89 (0.86–9.72)
Socioeconomic status	low	0.41 (0.12–1.47)	2.64 (0.69–10.12)
	medium/high	1.00	1.00
Practicing exercise (sports)	yes	1.00	1.00
	no	0.54 (0.16–1.81)	1.20 (0.29–4.79)
Type of RA	monoarticular	1.00	1.00
	polyarticular	**1.45 (1.22–1.72)**	NC
Remission	yes	1.12 (0.36–3.47)	0.80 (0.18–3.48)
	no	1.00	1.00
Erosive RA	yes	1.53 (0.45–5.26)	**1.34 (1.10–5.29)**
	no	1.00	1.00
Swelling	yes	0.84 (0.24–2.94)	0.76 (0.09–6.45)
	no	1.00	1.00
Pain	yes	0.63 (0.17–2.28)	0.35 (0.02–5.27)
	no	1.00	1.00
Morning stiffness	yes	0.95 (0.27– 3.30)	**2.22 (1.16–29.5)**
	no	1.00	1.00
Periodontitis	yes	1.13 (0.43–3.70)	0.52 (0.10–4.20)
	no	1.00	1.00
Stage of periodontal disease	I and II	1.00	1.00
	III and IV	**1.48 (1.20–1.90)**	NC
Alternative therapies	yes	**0.28 (0.19–0.42)**	NC
	no	1.00	1.00

OD – odds ratio; *CI* – confidence interval; NC – not calculated; † regression logistic model. All the variables presented in the table were included in the adjusted model.

Discussion

The main findings of this study indicate that the majority of surveyed RA patients report a low overall OHIP-14 score resulting in a low impact of oral health on their HRQOL (or a moderate/good HRQOL). However, there were differences with respect to some sociodemographic, general health, oral, and RA clinical variables. The dental variables that were most related to the OHRQOL dimensions were the PD stage, the number of teeth, and BOP. In a similar study, these variables were also significant.[26] In RA patients, the most related variable was RA type, defined by the number of affected joints and morning stiffness, a symptom that has been reported to impact the quality of life and a patient's optimism.[27]

Regarding age, despite the fact that people under 60 years of age report similar results in terms of OHIP-14 prevalence, those aged 60 and over had the highest scores regarding the extent and severity to which the OHRQOL had an impact on the oral health-related quality of life. This is explained by the physical deterioration in dental tissues due to increasing age and the physical impairments caused by RA.[28,29] These physiological changes can cause the perception of the OHRQOL to be diminished due to the presence of bleeding, tooth mobility, pain, occlusal trauma, as well as the use of certain medications, among others. Since oral diseases can spread and exacerbate systemic diseases or vice versa, cause discomfort, and increase the stigma experienced, it can have a potentially profound effect on behavior.[30]

In addition, the whole population being studied reported family support in handling RA, which had a low impact on the OHIP-14. A study carried out on RA patients in Serbia showed that the predictive values for suffering anxiety and fatigue were tied to the need for help and attention from other people as well as educational level.[31] A social gradient is observed in relation to the level of education of RA patients, with higher scores being observed in those with lower levels of education. Likewise, the lower class has the lowest quality of life, followed by the middle class. This has to do with the educational level and access to information regarding general and oral health, as well as the ability to understand instructions given by medical providers.[31] This can alter the ability to access health services, treatments, and programs offered by local institutions, especially for this particular population. These sociodemographic characteristics shape the way patients must address the disease process.[32] The lack of employment also makes the population in this study feel vulnerable in terms of their OHRQOL. This aspect concerns economic income and the possibility of having the resources to travel to the places where health care is provided. The degree of RA activity, quality of life, and functional capacity are mutually related to the work performance of the population studied. We found that the strongest association was with functional capacity.[33]

People who do not regularly exercise showed a higher prevalence of the OHRQOL impact compared to those who do exercise. This may be related to the fact that physical activity in RA patients improves the symptoms of the disease, which can influence general well-being and, in turn, be reflected in the perception of oral health and the possibility of self-care. A study conducted in Sweden resulted in a decrease in pain, an increase in quality of life, and greater self-efficacy in arthritis patients who participated in a supervised exercise and education program.[34]

As for people who have other systemic diseases such as diabetes, high blood pressure (HBP), and osteoporosis, the reported scores were similar to those who do not have any other systemic disease. This may be because the diseases are controlled at the time of consultation and treatment of RA, which changes the perception of oral health. In Chico's study, 59.0% of RA patients had HBP and in our study it was 32.2%.[35] In a study similar to this one, 95% of RA patients used non-biologic DMARDs and 20% used biologic ones. In the current study, the scores of patients on DMARDs were very similar to those not receiving DMARDs, since medication consumption controls RA symptoms, this could be reflected in the perception of both general and oral well-being.[36]

The OHIP-14 impact prevalence is similar in those with and without PD and only causes a slight variation in the severity of the impact. The impact on the OHRQOL perception is evident in those who have stage III and IV PD, in which the prevalence, extent, and severity result in worse scores in the OHRQOL. Similar results are reported in Jordanian adults with characteristics similar to the patients of this study.[37] Disease activity dictates the symptomatology exhibited by a patient and affects the measured blood indicators. The greater extent and severity of the OHIP-14 impact are reported in those experiencing high RA activity. Comparable results have been reported by other researchers.[38] When inquiring if the patients received alternative treatment — apart from the conventional one — such as homeopathy, bioenergetics, and natural treatments, among others, we found that only one person answered in the affirmative. People who answered in the negative reported the highest impact scores on the OHIP-14. These results corresponded with a study using complementary therapies in RA treatment and show how supportive treatments tend to improve a patient's perception of their quality of life.[39]

With regard to RA, self-perception variables such as swelling, joint pain, and morning stiffness produce a higher impact on OHRQOL. It is also evident that the variables associated with OHRQOL impact in RA patients are erosive RA and perceiving morning stiffness, both being risk factors.[38] Results were similar to those who reported fatigue and depression. In a study carried out on women with RA, the tendency was to be more vulnerable and less optimistic when the presence of daily pain was experienced.[27,40]

When comparing the different dimensions of OHIP-14, similar results are found in people with and without PD. Our findings identified the variables that are most correlated with the OHIP dimensions were age, number of teeth present, and periodontal bleeding. Out of these, the number of teeth has the highest and most significant correlations with all dimensions, showing an important biological gradient, meaning the lower the number of teeth, the greater the effect on the different dimensions. The previous results significantly differ from those of one study showing the effect of the different dimensions and global score of oral diseases and health-related quality exhibited by RA patients.[40] On the other hand, the more periodontal bleeding, the higher the impact on the dimensions of physical, psychological, social disability, and handicap. Also, the older the age, the higher the impact on physical and psychological disability in patients. Mühlberg's results are quite similar, demonstrating that RA patients showed a worse HRQOL than patients without RA, advocating for more intensive care of dental, medical, and psychological factors.[26]

As strengths of this research, it is important to mention the contribution it makes to the topic of oral health and quality of life in RA patients because few studies establish a relationship and comparison between these variables, which are crucial for the comprehensive treatment of people with this disease. We are aware that the use of non-probability sampling poses a limitation to our study since the results cannot be generalized as parametric estimates.

Follow-up studies are suggested to obtain information on changes over time and provide causality results to guide the comprehensive treatment of patients. Concerted efforts between rheumatologists and dentists in the management of oral health are recommended.

Considering the importance of studying the relationship between systemic conditions and oral health and their impact on quality of life, further research could identify other important aspects and determinants. For instance, studies should focus on joint disorders such as temporomandibular joint disorders in patients with PD and RA and their influence on the quality of life using qualitative and mixed methods approaches. Finally, studies focused on other oral manifestations of systemic diseases and mental health problems in patients with oral diseases could be useful in recognizing the impact of these pathologies on HRQOL and OHRQOL.

Conclusions

The findings of this study show the impact of oral health on a RA patient's quality of life. Differences regarding sociodemographic, clinical, and self-perception variables were found according to the prevalence, extent, and severity indicators of the OHIP-14. The dimensions with the greatest impact on the patient's quality of life were physical pain, psychological distress, and disability, both for patients with PD and for those without it. Multivariate models showed that some factors and conditions have more influence on the OHRQOL of these patients, for example, RA type and periodontal status are related to worse indicators. Based on these results, the establishment of epidemiological surveillance systems targeting oral health and systemic conditions could improve the monitoring of these patients and contribute to their general, social, and health well-being to help improve their quality of life.

Ethics approval and consent to participate

The protocol of this study was reviewed and approved by the Institutional Review Board at the Faculty of Dentistry of the University of Antioquia, Medellín, Colombia (Act 05-2016). Signed informed consent forms were collected prior to the initiation of the research 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.

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Original paper

Comparison of the pharyngeal airway in snoring and non-snoring patients based on the lateral cephalometric study: A case–control study

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Abstract

Background. Normal airways are a key factor during the craniofacial growth of the young. Therefore, sleep-disordered breathing (SDB) without treatment can have harmful consequences for development and health.

Objectives. This study aimed to evaluate the cephalometric characteristics in non-snoring individuals and snoring subjects, and investigate differences in the pharyngeal airway space between the 2 groups.

Material and methods. This case–control study included 70 patients aged over 18 years, selected from a radiology center. The patients were divided into 2 groups: case (35 patients with a history of habitual snoring); and control (35 healthy patients). The Berlin sleep questionnaire was administered to the parents of the patients. The nasopharyngeal airway was measured according to the analysis of Linder-Aronson (1970), and 4 indices were measured and analyzed in each of the lateral cephalometric radiographs.

Results. No statistically significant differences were observed in the pharyngeal measurements between the 2 groups, although all means in the control group were higher than in the experimental group. However, there was a significant relationship between gender and the Ba-S-PNS and PNS-AD2 indices.

Conclusions. Although the patients with nocturnal snoring had smaller airway dimensions, their pharyngeal measurements were not significantly different from the control group.

Keywords: snoring, obstructive, cephalometry, sleep apnea, pharynx

Introduction

The normal development and extension of the pharyngeal airway are key factors in craniofacial growth.[1] As a result, sleep-disordered breathing (SDB) in the absence of treatment may have harmful consequences for health.[2] However, the epidemiological studies on apnea prevalence in the general population are inconsistent (3–7% in males and 2–5% in females).[3]

The primary causes of snoring include narrowing of the upper respiratory tract due to nasal septum deviation, adenoid hypertrophy, tonsillitis, obesity (body mass index (BMI) >27 kg/m^2), edema secondary to inflammation of the oral cavity, sex (more common in males), and possibly genetics.[4]

A linear correlation exists between obesity and obstructive sleep apnea (OSA), with the precipitation of fat in the upper respiratory tract narrowing the airways and decreasing muscular activity, which leads to cyclic hypoxia and apnea.[5]

Snoring can lead to daytime sleepiness, increased risk of cardiovascular diseases,[6] and pregnancy-induced hypertension,[7] and continuous snoring may progress to OSA. This potential progression in complications requires specialist attention for diagnosis and treatment.[4] In preschool children, snoring has been reported in approximately 10% of the population, with OSA occurring in around 0.7−2.9%.[2] According to a review by Benjafield et al., 936 million (95% confidence interval (*CI*): 903–970) adults aged 30–69 years reported mild to severe OSA, and 425 million (95% *CI*: 399–450) adults aged 30–69 years reported moderate to severe OSA.[8]

OSA is a common disorder characterized by a relative or complete collapse of airways during sleep,[9] which can increase airflow resistance and stop breathing for 10 seconds or more and has significant medical and psychosocial effects on children and adults.[10] Apnea refers to the complete discontinuity of air for at least 10 seconds, while hypopnea suggests a partial reduction in the oronasal airflow and decreased oxygen saturation of oxyhemoglobin. OSA can result from various combinations of anatomical and pathophysiological features, some of which may be influenced by genetic factors.[11] Various characteristics reported for patients with OSA include a longer and thicker soft palate, decreased width of the oral or nasal airway,[12] increased thickness of the soft palate, a retruded chin, maxillary retrusion,[13] an increased craniofacial angle,[8] micrognathia,[14] and differences in the position of the hyoid bone.[15]

The prevalence of OSA among African Americans appears to be higher than in Caucasians.[16] Meanwhile, the prevalence of OSA in Asian populations is similar to that of Caucasians, though the OSA severity is higher.[17]

According to Morsy et al., 82% of men and 93% of women with moderate to severe OSA are never diagnosed.[18] Some studies have also shown a correlation between bruxism and OSA.[19,20]

Lateral cephalometric radiography is a static imaging technique that provides data that can be reproduced in detail and focuses on the anatomical characteristics of upper airways.[4] Generally, a lateral cephalogram is used in individuals with obstructive symptoms as a screening tool for assessing the upper airway morphology and craniofacial pattern, identifying individuals at risk of SDB, and studying the therapeutic effects of treatments.[2]

Definitive diagnosis of OSA requires polysomnography, though it is time-consuming and expensive. Cephalometric analysis is widely used as a diagnostic procedure in patients with OSA and for evaluating the effects of therapeutic interventions such as oral applicators.[15] Evidence-based medical assessment suggests that locating the obstruction should be the primary goal when researching sleep disorders.[21] However, no single assessment method is ideal, although lateral cephalometry may provide valuable information for the diagnosis, treatment, and screening of patients. Since individuals with narrow airways and craniofacial anomalies may be at increased risk of OSA and hypopnea syndrome, the use of a lateral cephalogram can play an important role in the early diagnosis and treatment of these patients.[12]

The present study aimed to compare pharyngeal airways in patients with and without snoring and to assess craniofacial features in individuals with SDB and snoring. The hypothesis was that patients with SDB would have large deviations from normal.

Material and methods

This case–control study selected participants from patients referred by their dentist for lateral cephalometric radiography for orthodontic treatment at a private radiology center in Qazvin, Iran. Initially, the parents of patients responded to the Berlin questionnaire.[22–24]

The Research Ethics Board at the Qazvin University of Medical Sciences, Iran, approved the study (IR.QUMS.REC.1396.145), which followed the guidelines of the Declaration of Helsinki. The questionnaire, developed at the Conference on Sleep in Primary Care in Berlin, Germany, in 1996, is a validated tool used to identify individuals at risk of OSA in primary and some non-primary care settings.[18] The Berlin questionnaire contains 11 questions in three categories. The 1st category comprises 5 questions regarding snoring, witnessed apnea, and the frequency of such events. The 2nd category includes 4 questions addressing daytime sleepiness, with a sub-question on drowsy driving. The 3rd category comprises 2 questions on a history of high blood pressure (>140/90 mmHg) and BMI > 30 kg/m^2. Categories 1 and 2 are considered positive if there are 2 positive responses to each category, while category 3 is regarded positive with a self-report of high blood pressure and/or BMI > 30 kg/m^2. The patients were scored as being at high risk of having OSA if scores were positive for 2 or more of the 3 categories. Those patients who scored positively for 1 category were identified as being at low risk of having OSA.[25]

Exclusion criteria included a history of previous orthodontic treatment, <8 years of age, known upper airway anomalies, chronic or recurrent infections (for example, tonsillitis or sinusitis), asthma, smoking, BMI > 90 kg/m^2, and systemic diseases, especially diabetes and hypertension. In addition, patients with skeletal class I occlusion with an A point, nasion, and B point (ANB) angle of 2–4° were included. The weight and stature were measured upon medical examination to calculate BMI by dividing weight (kg) by height squared (m^2).

The sample size was calculated as 34 patients in each group, according to a study by Kurt et al.,[10] by considering α = 0.05, P (statistical power) = 90% and d = 1.6%. Seventy patients participated in the study and were divided into 2 groups to compare pharyngeal airways, with 35 participants (18 males and 17 females) who had a history of habitual snoring of >6 months in the experimental group and 35 healthy participants (16 males and 19 females) in the control group.

Lateral cephalometric radiographs were taken with the participants in a standing position, with the teeth at maximum intercuspation, the lips in a relaxed position, and the head in the natural position, with the Frankfurt horizontal plane parallel to the ground. A 2-millimeter aluminum filter was used to visualize the nasal pyramid.[26] The nasopharyngeal airway was measured according to the analysis of Linder-Aronson (1970),[27] and 4 indices were measured in each lateral cephalometric radiograph.

Ba-S-PNS is used to determine the hard palatal and soft palatal horizontal position and the cause of airway obstruction, while nasopharyngeal airway adenoid-1 (PNS-AD1) is used for screening the airway obstruction, which is the pharyngeal diameters at the levels of the adenoids. PNS-AD2 is used for screening the airway obstruction and is measured as the distance from PNS to the nearest adenoid tissue on a perpendicular line from PNS to sella-basion (S-Ba). The PTV distance to the adenoid (PTV to AD) is used for airway obstruction screening, using a distance of 5 mm above the PNS and on the PTV to the nearest adenoid tissue (Fig. 1).

A radiologist and a dentist measured the indices for each patient separately. Subsequently, the mean of the variables associated with the upper airway was determined. Observations were repeated 2 weeks later, and the results were compared between the 2 stages. An independent-sample *t* test assessed intra-observer variations, with no significant differences found in the values of the observed parameters.

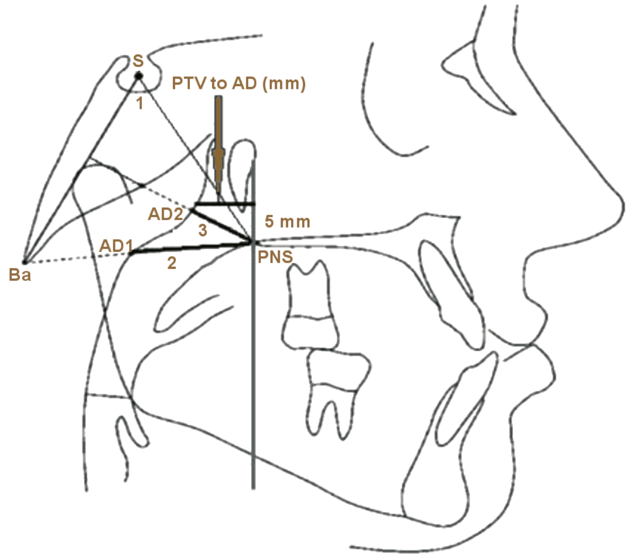

Fig. 1. Nasopharyngeal airway measurements

Ba – basion; S – sella; PNS – posterior nasal spine; AD1 – adenoid 1: the nearest adenoid tissue measured over the line of PNS-Ba; AD2 – adenoid 2: the nearest adenoid tissue measured on the perpendicular line from S to Ba; PTV – vertical pterygoid plane.
Ba-S-PNS angle [°]: it is used to determine the hard palatal and soft palatal horizontal position, as well as the cause of airway obstruction; PNS-AD1 [mm]: line 1 is used for screening the airway obstruction (it is the pharyngeal diameter at the level of the adenoids); PNS-AD2 [mm]: line 2 is used for screening the airway obstruction (it is the distance from PNS to the nearest adenoid tissue on a perpendicular line from PNS to S-Ba); PTV to AD [mm]: it is used for airway obstruction screening (the distance is measured 5 mm above PNS on PTV to the nearest adenoid tissue).

Statistical analysis

Data analysis employed IBM SPSS Statistics for Windows, v. 25.0 (IBM Corp., Armonk, USA). Data was expressed as mean and standard deviation (M ±SD). The Kolmogorov–Smirnov test determined the normality of data distribution. All variables were normally distributed except for age. The cephalometric measurements in the 2 groups were compared using the analysis of variance (ANOVA), and Levene's test confirmed the equality of variance. The independent-sample *t* tests detected significant differences in the craniofacial and airway space measurements between the 2 groups. Statistical significance for all tests was set at $p < 0.05$ (2-tailed).

Results

The mean age of the participants was 10.4 years, which ranged from 8.1 to 12.9 years. The distribution of age, height and weight was the same among the studied groups. Based on the Berlin questionnaire, 19 samples (54.28%) were low-risk, and 16 (45.72%) were high-risk in the experimental group. The BMI values in the experimental and control groups were 43.0 ±5.3 kg/m^2 and 39.0 ±8.7 kg/m^2, respectively, with no significant difference between the 2 groups.

Table 1 presents the mean values for Ba-S-PNS, PNS-AD1, PNS-AD2, and the distance between PTV and adenoid. There were no significant relationships between the 4 indices in patients with and without snoring. Furthermore, the independent-samples *t* tests showed no relationship between indices in males and females with and without snoring (Table 2 and 3).

Table 4 shows the relationship between sex and radiographic indices. There was a significant relationship between sex and the Ba-S-PNS index in individuals with and without snoring. Also, a significant relationship was found between sex and the PNS-AD2 index in patients with snoring. However, the relationships between sex and the other indices in patients with and without snoring were not significant.

Table 1. Comparison of the cephalometric pharyngeal airway measurements in patients with and without snoring

Index	Snoring	*M* ±*SD*	Range	*p*-value
Ba-S-PNS [°]	no	61.15 ±5.90	47.72–73.67	0.240
	yes	59.58 ±5.14	48.71–69.26	
PNS-AD1 [mm]]	no	32.58 ±7.50	16.13–43.20	0.500
	yes	31.46 ±6.25	12.21-43.64	
PNS-AD2 [mm]	no	27.21 ±7.03	15.14–41.91	0.670
	yes	26.50 ±6.75	11.43–40.70	
PTV to AD [mm]	no	18.40 ±7.56	4.36–32.94	0.540
	yes	17.39 ±6.32	1.58–32.09	

M – mean; *SD* – standard deviation.

Table 2. Comparison of the cephalometric pharyngeal airway measurements in male patients with and without snoring

Index	Snoring	*M* ±*SD*	*p*-value
Ba-S-PNS [°]	no	58.57 ±4.56	0.500
	yes	57.49 ±4.67	
PNS-AD1 [mm]]	no	32.68 ±7.58	0.960
	yes	32.78 ±4.98	
PNS-AD2 [mm]	no	27.10 ±7.16	0.470
	yes	28.80 ±6.48	
PTV to AD [mm]	no	19.61 ±8.41	0.850
	yes	19.17 ±5.74	

Table 3. Comparison of the cephalometric pharyngeal airway measurements in female patients with and without snoring

Index	Snoring	*M* ±*SD*	*p*-value
Ba-S-PNS [°]	no	63.32 ±6.18	0. 410
	yes	61.79 ±4.79	
PNS-AD1 [mm]	no	32.50 ±7.41	0.320
	yes	30.06 ±7.26	
PNS-AD2 [mm]	no	27.30 ±7.12	0.160
	yes	24.07 ±6.31	
PTV to AD [mm]	no	17.37 ±6.82	0.410
	yes	15.51 ±6.52	

Table 4. Relationship between sex and the cephalometric indices

Index	Snoring	Sex	*M* ±*SD*	*p*-value
Ba-S-PNS [°]	no	M	58.57 ±4.56	0.016*
		F	63.32 ±6.18	
	yes	M	57.49 ±4.67	0.011*
		F	61.79 ±4.79	
PNS-AD1 [mm]	no	M	32.68 ±7.85	0.940
		F	32.50 ±7.41	
	yes	M	32.78 ±4.98	0.200
		F	30.06 ±7.26	
PNS-AD2 [mm]	no	M	27.10 ±7.16	0.930
		F	27.30 ±7.12	
	yes	M	28.80 ±6.48	0.036*
		F	24.07 ±6.31	
PTV to AD [mm]	no	M	19.61 ±8.41	0.390
		F	17.37 ±6.82	
	yes	M	19.17 ±5.74	0.080
		F	15.51 ±6.52	

M – male; F – female; * statistically significant ($p < 0.05$).

Discussion

Possible causes of OSA are anatomical narrowing of the upper airway as a consequence of alterations in the craniofacial morphology or soft tissue enlargement, sleep posture, age, male gender, nasal obstruction, and adipose tissue in the pharynx.[25] However, the evaluation of airways in snoring patients has not been the focus of much attention.[2] Therefore, this study used lateral cephalograms to investigate the upper airway structures in snoring and non-snoring patients.

The present study found no statistically significant differences in the indices between the two groups. However, the mean airway indices were numerically higher in all non-snoring individuals, which is consistent with the findings reported by Pirila-Parkkinen et al., who compared snoring and non-snoring individuals in terms of PNS-AD1 and PNS-AD2 indices.[2] In their study, OSA patients showed the most deviations from the control group in terms of cephalometric findings compared to the other subgroups of obstructive sleep disorders. More specifically, there was a significant difference in PNS-AD1 between the OSA and the control groups. They also observed that airway obstruction was more severe in the OSA group. Also, the findings of the present study concur with a study by Kurt et al. comparing snoring and non-snoring patients in terms of the PNS-PPW1 index (upper pharyngeal space).[10]

In the present study, the difference between males and females only reached statistical significance for the Ba-S-PNS index ($p = 0.016$). Specifically, the mean value of this index was higher in non-snoring females than in non-snoring males, but the means of the other three indices in both sexes were almost the same. A study by Daraze et al. on healthy Lebanese participants showed a significant difference between sexes regarding the PNS-AD1 index. This contrast with the present study could be attributed to the difference in sample size and ethnicity. However, Daraze et al. reported no significant difference in the PNS-AD2 index between the two sexes.[27]

A longitudinal study by Peppard et al. showed that an increase in weight of 10% (relative to stable weight) predicted a 6-fold increase in the odds of developing moderate to severe SDB, and a 32% increase in apnea-hypopnea index (AHI) during a follow-up period of four years, while a weight loss of 10% led to a 26% reduction in AHI.[21]

Hou et al. reported that deviations in craniofacial morphology are more common in Chinese patients with severe OSA,[28] whereas Rose et al. did not establish a direct relationship between cephalometric findings and OSA severity.[29]

The current study found a statistically significant difference in the Ba-S-PNS and PNS-AD2 indices between snoring males and females. However, no significant relationship was established between these two groups regarding the other two indices. In addition, Pirila-Parkkinen et al. showed no significant difference between sex and pharyngeal airway indices in patients with obstructive sleep disorders.[2]

Studies have considered the relationship between airways and anatomical structures and the type of malocclusion. According to Dastan et al., the upper airway volume in sub-groups did not show a significant difference.[30] Therefore, these indicators were not evaluated separately in this study. However, some studies have shown that the vertical height of the face can affect airway volume.[30–32]

Patients with hypertension and diabetes were excluded because, according to studies, the risk of bruxism increases with these disorders, and bruxism can cause airway disorders.[33,34]

According to Li et al.,[35] orthodontic treatment interventions could change the dimensions of the upper airways, whereas Abdalla et al. did not confirm this finding.[36] Numerous other studies demonstrated the corrective effect of combined surgical and orthodontic treatments on the airway space.[35–37] Therefore, the present study excluded patients with a history of orthodontics.

This study examined the nasopharyngeal airway exclusively. Other factors, such as the oropharyngeal airway, hyoid bone, and nasal cavity anatomy, will be the subject of subsequent studies to evaluate their relationship with the nasopharyngeal airway.

Limitations of this study included difficulty in accessing OSA patients, expensive and time-consuming processes, the small sample size, and the assessment of two-dimensional radiographs for airway measurements. Future studies will overcome these limitations by initially employing three-dimensional imaging airway segmentation and volume measurements in the subsequent research phase.

Conclusions

The present study found no significant differences in the studied indices between the snoring and non-snoring subjects. Therefore, the airway anatomy was not significantly different between these two groups. However, the absence of no significant differences between the two groups could be attributed to the higher proportion of low-risk subjects than high-risk subjects in the experimental group.

Ethics approval and consent to participate

The Research Ethics Board at the Qazvin University of Medical Sciences, Iran, approved the study (IR.QUMS.REC.1396.145). Written informed consent was obtained from all study participants prior to the initiation of the research 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.

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Original paper

Degenerative changes of the mandibular condyle in relation to the temporomandibular joint space, gender and age: A multicenter CBCT study

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Abstract

Background. Cone-beam computed tomography (CBCT) is used to provide multiplanar views of the temporomandibular joint (TMJ) bone components as well as TMJ pathologies without superposition, magnification or distortion.

Objectives. The study aimed to analyze degenerative changes in the condylar surface, and their relationship with patient age and gender, and the TMJ space measurements by using CBCT images.

Material and methods. A total of 258 individuals were retrospectively analyzed. The degenerative bone changes of the condylar head were evaluated and classified on the right and left sides. The shortest distances from the anterior, superior and posterior parts of the condylar head to the glenoid fossa were measured to represent the TMJ space. Univariate and multivariate logistic regression analyses then evaluated the effect of age and gender on the presence of degenerative changes.

Results. Condylar flattening was most frequently observed (413 TMJs, 53.5%). However, the presence or absence of the change types did not differ according to the sides. The mean values of the TMJ space measurements on the right and left sides were narrower in the group with changes than in the group without changes. Nonetheless, no statistically significant difference in the TMJ space was found between the groups ($p > 0.05$).

Conclusions. An increased risk of radiographically detectable degenerative alterations in left TMJs was detected for males and for increasing age. Degenerative changes in the condylar surface may affect the dimensions of the TMJ space.

Keywords: temporomandibular joint, cone-beam computed tomography, mandibular condyle, degeneration, age

Introduction

The temporomandibular joint (TMJ) is located between the mandibular condyle (the inferior part of the TMJ) and the glenoid fossa of the temporal bone (the superior part of the TMJ) and is considered to be the most complex articular system in the human body. The TMJ can move on different orthogonal planes with a balanced, dynamic, and complex mechanism.[1,2]

The optimal condylar head position in the glenoid fossa is one of the most discussed subjects in dentistry. Despite the occlusion being detected directly intraorally, the condylar position in the fossa cannot be interpreted with the naked eye. However, the joint space dimensions could determine the ideal position of the condyle in the glenoid fossa. Indeed, the joint space is a commonly used radiological term to describe the radiolucent region between the condylar head and the temporal bone.[3]

Temporomandibular disorder (TMD) is a general term to describe conditions affecting the masticatory muscles, the TMJ, and related structures, either individually or all at once.[4] Furthermore, TMD is often associated with disc displacement and degenerative changes that affect soft and/or hard tissues of the TMJ.[5] Degenerative bone changes in the TMJ are more common in the mandibular condyle than in the glenoid fossa or the articular eminence, which includes various degrees of flattening, erosion, osteophyte formation, sclerosis, and pseudocysts.[6–9] It is essential to identify these pathologies, as in-depth knowledge of bone changes is necessary for the accurate diagnosis of disease-related dysfunctions and appropriate treatment planning.[10]

Radiographic examination is necessary for the proper diagnosis of TMD,[11] with many imaging techniques and diagnostic modalities used to examine the joint. TMJ radiography assesses the degree of disease, progress, and treatment response.[12] Meanwhile, panoramic radiography, conventional tomography, helical or multi-slice computed tomography (MSCT), and cone-beam computed tomography (CBCT) are used to evaluate the bony components, while magnetic resonance imaging (MRI) can examine the soft tissues, such as the disc or capsule of the TMJ.[13]

CBCT is reported to be more useful for detecting condylar surface pathologies than conventional imaging modalities. Indeed, the superimposing of structures in conventional radiography could result in misdiagnosis due to poor radiographic evaluation.[6,7] The Diagnostic Criteria for TMD (DC/TMD) indicate that CT imaging is required for determining degenerative joint disease.[14] However, the relatively high radiation dose and cost limit CT use for TMJ examination.[15] Recently, CBCT imaging has been used as an alternative method for evaluating the TMJ bone components in all three dimensions without superimposition and structural distortion.[16]

This study aimed to analyze degenerative condylar changes and to determine the possible relationship between degenerative condylar changes and age, sex, side (right/left), and joint space distances.

Material and methods

Study design

The local Research Ethics Committee approved the protocol of this multicenter study (No.: 14/3 and Ref.: 36290600/124), and the study complied with the 1964 Declaration of Helsinki ethical guidelines.

The digital images of patients who underwent CBCT examination at the Dentomaxillofacial Radiology Departments of Ankara University, Zonguldak Bülent Ecevit University, and the Near East University for various reasons from 2013 to 2019 were retrospectively analyzed. The CBCT records of the bilateral TMJs of 258 individuals (148 females and 110 males) aged 8–82 years (mean age: 39.81 ±15.6 years) were evaluated.

The study included CBCT images with completely visible bilaterally TMJs, all posterior teeth present, degenerative condylar changes in at least one TMJ, and cases without a history of systemic diseases. Exclusion criteria for the study included low-quality images, images with radiologic signs of metabolic bone disease, noticeable periodontal diseases, prosthetic restorations, a history of orthodontic treatment, disorders that may affect joint morphology such as rheumatoid arthritis, a history of surgery, trauma, cyst, or tumor in the maxillofacial region, and congenital or developmental disorders.

Imaging procedures

All CBCT scans were performed based on local imaging protocols. The technical parameters and dedicated software of the CBCT units used are presented in Table 1.

CBCT evaluation

A single informed and calibrated specialist in dental radiology (CG with 6 years of experience, Mİ and SA with more than 10 years of experience) analyzed images at each center. A standard positioning was defined before the evaluation, and the examiners placed each image in that position.

Adjustments to the CBCT images ensured that the vertical reference line ran through the patient's midsagittal plane in the axial and coronal views. The hard palate was oriented in the sagittal view so that the horizontal reference line was positioned through the anterior and posterior nasal spine. The tool "TMJ module" was activated. When the largest mediolateral width of the condylar process was visible in the axial plane, paracoronal and

Table 1. Technical parameters and software programs of the cone-beam computer tomography (CBCT) devices used

		CBCT device		
		ProMax®3D Max	NewTom 3G®	Veraviewepocs 3D®
Technical parameters	FOV size	130 × 55 mm, 230 ×160 mm	12-inch	40 × 80 mm
	voxel size	0.200 mm^3, 0.400 mm^3	0.300 mm^3	0.125 mm^3
	kVp	96	120	90
	mA	8–12	3–5	5
	scan time [s]	9–15	–	9.4
Software program		Romexis 3.7	NNT 3.0	3D Tomo X
Vendor		Planmeca Oy	Quantitative Radiology	IORB
Monitor		NEC MultiSync 21.3-inch flat-panel 2048 × 2560 pixel resolution	Nio Color 3MP 2048 × 1536 pixel resolution	EIZO RadiForce MS230W 23-inch LCD monitor
Vendor		Sharp NEC Display Solutions	Barco	Eizo Nanao Corporation

FOV – field of view.
ProMax 3D Max by Planmeca Oy, Helsinki, Finland; NewTom 3G by Quantitative Radiology, Verona, Italy; Veraviewepocs 3D by J. Morita MFG Corp., Kyoto, Japan.

parasagittal cross-sectional slices were obtained for qualitative and quantitative analysis (Fig. 1).

Records for each patient included age, sex, degenerative alteration types, and TMJ space measurements. Bone changes were detected in at least two consecutive sections to avoid misinterpretations, and images were excluded when all examiners doubted the classification.

Diagnostic classification of degenerative changes in the condylar surface

Previously reported definitions of flattening (Fig. 2A), sclerosis (Fig. 2B), erosion (Fig. 2C), osteophytes (Fig. 2D), subchondral cyst (Ely's cyst) (Fig. 2E), ankylosis (Fig. 2F), and loose joint bodies (Fig. 2G), evaluated degenerative changes of the condylar head.[17,18]

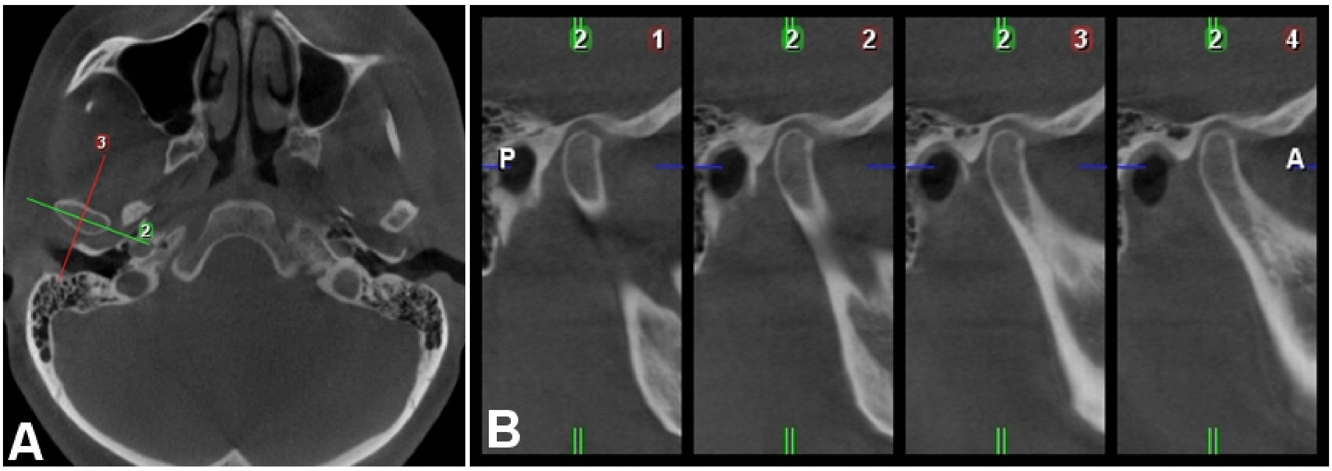

Fig. 1. A – axial image showing the largest mediolateral length of the condyle; paracoronal slice of the condyle (green line) and parasagittal slice of the condyle (red line); B – parasagittal reconstruction of the temporomandibular joint (TMJ) in maximum intercuspation

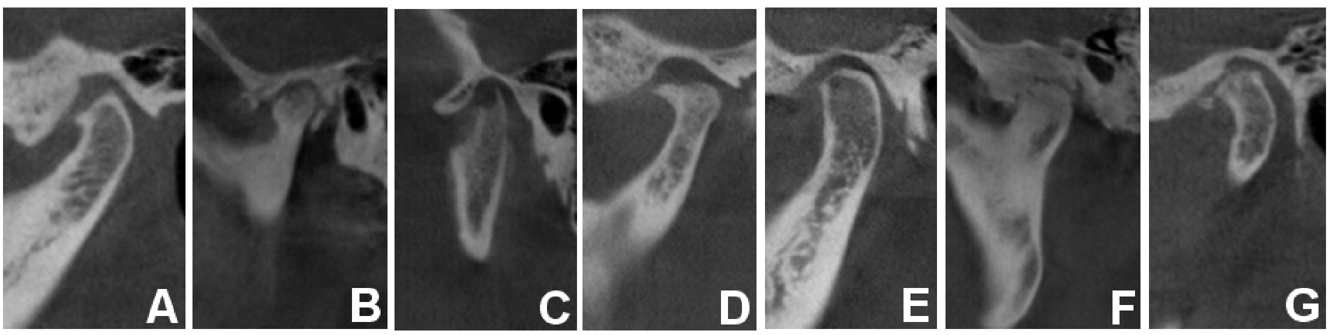

Fig. 2. Classification of degenerative bone changes

A – flattening; B – sclerosis; C – erosion; D – osteophyte; E – subchondral cyst; F – ankylosis; G – loose joint bodies.

The TMJs were classified into 2 groups: (1) without-change (when the condyles had a smooth, clear cortical bone surface); and (2) with-change (flattening, erosion, osteophytes, sclerosis, subchondral cyst, ankylosis, and/or loose joint bodies).

TMJ space measurements

The TMJ space calculations used dedicated software programs on the central parasagittal view, with a horizontal line parallel to the palatine plane used as the reference line. Linear measurements were made at selected points of the glenoid fossa and the condylar head at the shortest distances. All landmarks used for measurements are presented in Fig. 3.

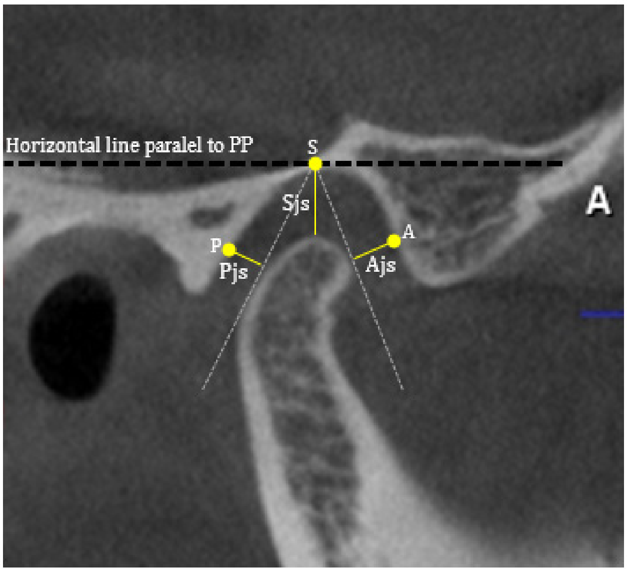

Fig. 3. Measurement of the joint spaces in the central parasagittal view

The distance between the most superior surface of the condylar head and the top point of the glenoid fossa (S) on the horizontal plane was measured as the Sjs. Two tangent lines (dashed lines) were drawn from the S point to the most prominent part of the anterior and posterior condylar head. The shortest distances between these points and the fossa wall (A and P) were measured as Ajs and Pjs, respectively. Ajs – anterior joint space; Sjs – superior joint space; Pjs – posterior joint space; PP – palatinal plane.

Statistical analysis

All statistical analyses employed SPSS Statistics for Windows, v. 11.5 (IBM Corp., Armonk, USA). Student's t test compared normally distributed variables between the TMJ groups, with the Mann–Whitney U test applied to non-normally distributed data. Meanwhile, McNemar's test assessed differences in the right and left sides between the 2 groups. Logistic regression analyses determined the relationship between the risk factors and the occurrence of degenerative bone changes. A p-value <0.05 was considered statistically significant.

Results

Table 2 shows the frequency distribution of degenerative alterations in condyle based on gender and side. Degenerative bone changes were detected in the 467 TMJs (232 TMJs-right and 235 TMJs-left, respectively). A total of 209 of the 258 cases had bilateral condylar bone changes, with the remaining 49 individuals showing unilateral bone changes (26 on the right side and 23 on the left side). According to McNemar's test, there was no significant difference in the degenerative change types between the right and left sides of the TMJ ($p = 0.668$).

Table 3 outlines the absence or presence of condylar bone alterations according to joint space measurements. The mean values of anterior joint spaces (Ajs) and superior joint spaces (Sjs) of the right TMJs, as well as Ajs, Sjs, and posterior joint spaces (Pjs) of the left TMJs were smaller in the with-change group than in the without-change group. However, there was no statistically significant association between the presence of degenerative changes and joint space measurements.

Table 2. Distribution of degenerative bone changes according to gender and side

Degenerative changes		Right side			Left side		
		F	M	Total	F	M	Total
Presence of degenerative bone changes*	without change	14 (9.5)	12 (10.9)	26 (10.1)	19 (12.8)	4 (3.6)	23 (8.9)
	with change	134 (90.5)	98 (89.1)	232 (89.9)	129 (87.2)	106 (96.4)	235 (91.1)
	Total	**148 (100)**	**110 (100)**	**258 (100)**	**148 (100)**	**110 (100)**	**258 (100)**
Types of degenerative bone changes**	Flattening	116 (51.1)	89 (55.3)	205 (52.8)	119 (54.8)	89 (53.3)	208 (54.2)
	Sclerosis	28 (12.3)	15 (9.3)	43 (11.1)	25 (11.5)	12 (7.19)	37 (9.64)
	Erosion	44 (19.4)	42 (26.1)	86 (22.2)	41 (18.9)	42 (25.1)	83 (21.6)
	Osteophyte	24 (10.6)	10 (6.2)	34 (8.76)	21 (9.68)	18 (10.8)	39 (10.2)
	Subchondral cyst	11 (4.8)	2 (1.2)	13 (3.35)	6 (2.76)	3 (1.8)	9 (2.34)
	Ankylosis	0 (0)	3 (1.9)	3 (0.77)	1 (0.46)	1 (0.6)	2 (0.52)
	Loose joint bodies	4 (1.8)	0 (0)	4 (1.03)	4 (1.84)	2 (1.2)	6 (1.56)
	Total	**227 (100)**	**161 (100)**	**388 (100)**	**217 (100)**	**167 (100)**	**384 (100)**

Data presented as number (percentage) (n (%)). * number of patients; ** number of temporomandibular joints (TMJs); F – female; M – male.

Table 3. Comparison of the temporomandibular joint (TMJ) spaces between the groups with and without changes

Joint space measurements [mm]	Right-side degenerative changes				*p*-value
	without changes		with changes		
	M ±*SD*	*Me* (min–max)	*M* ±*SD*	*Me* (min–max)	
Ajs	2.34 ±0.71	2.20 (1.44–4.16)	2.25 ±0.97	2.07 (0.57–7.77)	0.396[b]
Sjs	3.35 ±0.82	3.27 (1.80–5.34)	3.26 ±1.30	3.22 (0.72–10.19)	0.559[b]
Pjs	2.18 ±0.80	2.25 (0.57–4.88)	2.18 ±0.95	2.00 (0.60–8.05)	0.665[b]
Joint space measurements [mm]	**Left-side degenerative changes**				***p*-value**
	without changes		**with changes**		
	M* ±*SD	***Me* (min–max)**	***M* ±*SD***	***Me* (min–max)**	
Ajs	2.36 ±0.97	2.23 (0.89–4.47)	2.28 ±1.00	2.15 (0.47–7.73)	0.609[b]
Sjs	3.40 ±1.16	3.57 (1.20–5.58)	3.34 ±1.25	3.22 (0.30–7.20)	0.821[a]
Pjs	2.12 ±0.82	2.02 (0.89–4.10)	2.10 ±0.85	2.00 (0.57–6.99)	0.820[b]

M – mean; *SD* – standard deviation; *Me* – median; min – mnimum; max – maximum; [a] Student's t test; [b] Mann–Whitney *U* test.

Table 4 presents the prevalence of the degenerative change types on the right and left sides. The most common degenerative bone changes were flattening, erosion, and sclerosis, for the right and left sides. Meanwhile, ankylosis and loose joint bodies were the least common degenerative changes. The presence or absence of the change types did not differ between the left and right sides.

The univariate associations of degenerative bone changes with age and gender were analyzed. Increasing age and being male had a significant association, but only on the left side ($p = 0.004$ and $p = 0.022$, respectively) (Table 5). In the multivariate analysis, male gender and increasing age (odds ratio (*OR*): 4.37; 95% confidence interval (*CI*): 1.40–13.63; $p = 0.011$) had a significant association with left-sided degenerative changes (Table 6).

Table 4. Distribution and comparison of the type of degenerative changes according to side

	Type of change	present	Left TMJ				*p*-value
			no		yes		
			n	%	*n*	%	
Right TMJ	Flattening	no	13	24.5	40	75.5	0.731[a]
		yes	36	17.6	169	82.4	
	Sclerosis	no	194	90.2	21	9.8	0.471[a]
		yes	27	62.8	16	37.2	
	Erosion	no	133	77.3	39	22.7	0.824[a]
		yes	42	48.8	44	51.2	
	Osteophyte	no	196	87.5	28	12.5	0.576[a]
		yes	23	67.6	11	32.4	
	Subchondral cyst	no	236	96.3	9	3.7	0.523[a]
		yes	13	100.0	0	0.0	
	Ankylosis	no	253	99.2	2	0.8	1.000[a]
		yes	3	100.0	0	0.0	
	Loose joint bodies	no	248	97.6	6	2.4	0.754[a]
		yes	4	100.0	0	0.0	

n – number of TMJs; [a] McNemar's test.

Table 5. Univariate logistic regression analysis for the presence of degenerative bone changes

Variables (Reference)		β	*SE*	*p*-value	*OR*	95% *CI*
Right TMJs	Age	0.017	0.014	0.209	1.018	0.990–1.046
	Gender (M)	0.159	0.415	0.702	1.172	0.519–2.645
Left TMJs	Age	0.052	0.018	0.004*	1.053	1.017–1.090
	Gender (M)	1.300	0.568	0.022*	3.669	1.205–11.171

β –beta coefficient; *SE* – standard error; *OR* – odds ratio; *CI* – confidence interval; * statistically significant ($p < 0.05$).

Table 6. Multivariate logistic regression analysis for the presence of degenerative bone changes on the left side

Variables (Reference)	β	*SE*	*p*-value	*OR*	95% *CI*
Constant	−0.076	0.642	0.906	–	–
Age	0.057	0.018	0.002*	1.059	1.021–1.098
Gender (F)	1.474	0.581	0.011*	4.368	1.400–13.628

* statistically significant ($p < 0.05$).

Discussion

Osteoarthritis has been described as a low-inflammatory degenerative disorder that initially affects the articular cartilage and subcondylar bone, then causes destructive changes in the cortical and subcortical bone areas. Furthermore, osteoarthritis appears to be a common degenerative and destructive joint change due to primary and secondary trauma or other acute or chronic extreme conditions.[19]

Larheim and Westesson described osteoarthritis as prominent inflammatory findings on MRI, whereas osteoarthrosis refers to joints without such symptoms.[20] Osteoarthritis leads to bone deformation, including subcortical cysts, surface erosion, osteophytes, and sclerosis. However, osteoarthritis requires more specific and detailed diagnostic guidelines with the increasing use of CBCT.[18]

Several studies have reported the variable prevalence rates of structural TMJ alterations.[8,9,17,21–23] Koyama et al. examined condylar bone changes in 516 patients with TMD using helical CT and detected changes in 617 (63.7%) of 1032 joints.[9] Also, Koç found that 67.3% of the retrospectively evaluated patients presented one or more osseous TMJ changes on CBCT images.[21] Meanwhile, Pontual et al. examined the CBCT scans of patients with clinical symptoms or signs of TMJs and found TMJ bone alterations in 227 (71%).[22] Cho and Jung examined CBCT images for osteoarthritic changes of the TMJ in Korean children and adolescents and found that TMJ osteoarthritis was much higher in symptomatic cases (26.8%) than in asymptomatic cases (9.9%).[8] Moreover, Borahan et al. revealed osteoarthritic changes (17.9%) and developmental changes (2.6%) in the CBCT images of 795 patients.[17] Walewski et al. reported a higher prevalence (52.3%) of degenerative TMJ changes in asymptomatic groups,[23] with similar results presented by Krisjane et al. (42.7%).[24] These reported differences may be due to variations in patient selection, imaging techniques, or evaluation criteria. The current study detected osteoarthritic alterations in 91.1% ($n = 235$) and 89.9% ($n = 232$) of the left and right joints, respectively. Such high rates were achieved as all images had an osseous alteration on at least one side.

There are disagreements about whether clinical symptoms affect the radiographic evidence of TMJ osteoarthritis. Indeed, some studies have reported a poor correlation between TMJ osteoarthritis and signs and symptoms of TMD.[25,26] Wiese et al. did not find any relationship between radiographic findings and pain-related variables.[27] In contrast, Kurita et al. mentioned that TMJ pain was associated with mandibular function and osteoarthritic alterations in the articular surface.[28] Radiographic examinations have an essential role in evaluating bone changes in the TMJ. Some of these changes can be due to age-related remodeling mechanisms or physiological responses without clinical signs, as radiographs indicate the effect of a previous process and not ongoing processes.[29] Therefore, radiographic findings may not always correlate with the clinical situation. In this regard, the current study did not assess the clinical findings of the patients as it was designed to assess the types of degenerative changes of the condyle and to investigate the association between joint space and condylar alterations.

Some authors have reported a correlation between degenerative changes and increased age. Koç reported a significantly higher mean patient age with findings of osseous changes in the TMJ compared to those without any changes.[21] According to Pontual et al., advanced age is related to having more frequent degenerative bone changes, and bone alterations occur more commonly in females.[22] Alexiou et al. showed that older patients had more frequent bone changes than younger individuals.[30] Similarly, Borahan et al. reported increased TMJ degenerative changes in individuals over 60 years, which were more common in females than in males.[17] However, several studies found no association between age and osseous alterations.[22,23,30–32] Cho and Jung evaluated symptomatic and asymptomatic children and adolescents and found no significant difference between the children and adolescents.[8] Nonetheless, the older age group had a higher prevalence of degenerative changes. In addition, Crusoe-Rebello et al.[31] and Isberg et al.[32] concluded that there was no relationship between older age and the incidence of bone alterations. The present study found no correlation between age and degenerative changes in the right TMJ, whereas aging was a risk factor for condylar alterations on the left side. The difference may be related to an unbalanced distribution of biomechanical stress on TMJs caused by chewing side preferences. During mastication, bilateral usage is necessary, but unilateral predominance is a well-known and relatively consistent phenomenon.[33] However, due to its retrospective design, this study did not include information related to parafunctional activity and chewing side preferences.

It is widely accepted that females are more likely to develop TMDs, with several studies revealing more frequent osteoarthritic changes in female patients, which is mainly due to hormonal differences between genders.[21,22,28,29] In contrast, some authors found no gender-based differences.[23,24] In research by Cho and Jung, the prevalence of osteoarthritic changes was higher for males in the symptomatic group, while no significant gender differences were observed in the asymptomatic group.[8] The current study detected an increased risk of condylar degenerative changes in males with increasing age. However, the results may be associated with gender differences in willingness to seek help and may reflect the true predilection. Indeed, treatment-seeking rates may be lower in males than females, with the former only visiting the clinic at an advanced stage of the disease.

Borahan et al. reported a more common distribution of osteoarthritic changes on the right side, which could reflect individuals' chewing side preference.[17] However, Koç[21] and Pontual et al.[22] observed a higher prevalence of bone changes on left TMJs, but this difference was not significant. Similarly, this study showed no significant difference in degenerative changes between the sides.

In the current study, flattening, erosion, sclerosis, and osteophyte lesions predominated on both sides. These findings are in line with Güler et al., who scanned patients with bruxism using MRI.[34] A similar result was also outlined in a CBCT study by Alexious et al., who found that erosion, flattening, and osteophytes were predominant, but sclerosis was the least common lesion.[30] Meanwhile, Koç evaluated bone changes in the condylar head, articular fossa, and articular eminence and revealed more frequent osseous changes in the condyle than in temporal bone components.[21] In that CBCT study, flattening was the most common finding for condylar head and articular eminence, but erosion in the articular fossa was more frequent.[21] Indeed, flattening is the most frequently detected degenerative feature reported in the literature.[7,21,22,29,30,35] These results can be explained by the probability that flattening indicates an adaptive change, with the early finding of progressive disease or alteration secondary to internal derangement. Also, it can be related to the mechanical (masseter and temporal muscles) overloading of the TMJ.[22] However, the imaging findings reported from other studies disagree with this result. Cho and Jung concluded that the most frequent radiographic findings were erosion (15.6%) and sclerosis (5.4%) for the symptomatic and asymptomatic groups, respectively.[8] These authors also found that erosion was found more frequently in patients with pain and limited mouth opening. Nah et al. evaluated 220 TMJ patients using CBCT, and the most common finding was sclerosis (30.2%), followed by erosion (29.3%), flattening (25.5%), and osteophytes (8.0%).[18] Meanwhile, Mani and Sivasubramanian detected erosion as the most predominant change.[35] The discrepancy between findings may result from imaging technique differences, racial/ethnic diversity, age groups, and/or diagnostic criteria.

Structural changes may indicate different stages of degenerative joint disease. Erosion findings can correspond to acute changes and is a sign of the TMJ being in an unstable state and changes occurring on the bone surface. Flattening and osteophyte formation are detected in the advanced stage of the disease. Indeed, osteophytes, flattening, and sclerosis formations indicate the adaptation of the body to repair the joint.[36]

Panchbhai reported different TMJ space analysis methods and their comparisons and associations with other parameters in a review article.[37] Meanwhile, Koç classified measurements of less than 1.5 mm in all directions (anterior, superior, and posterior) of the TMJ as reduced joint space.[21] Furthermore, the research found a statistically significant difference between the mean age and joint space narrowing. It was concluded that joint narrowing accompanied osseous changes in the TMJ.[21] The current study found narrower joint space values in the with-change group than in the without-change group, though the difference was not statistically significant. Similarly, Tsuruta et al. found no significant difference in Ajs or Pjs measurements, neither between the presence and absence of bone change nor between the bone-change subgroups.[38] In addition, the joint space measurements were not categorized as continuous variables since there was no clinical or prognostic reason for this.

It has been reported that the TMJ space could be regarded as a diagnostic metric for TMD.[39] However, the bone changes that might occur are not very predictable in an altered joint space or vice versa. To better understand this relationship, the disc position must be one of the parameters evaluated in future studies.

A potential limitation of the study is that the findings do not explain the longitudinal alterations in the condyle due to the study's observational design. In addition, the observational design limits the degree of the cause-and-effect relationships. Furthermore, degenerative lesion-type frequency without clinical symptoms was described due to the study's retrospective design. Moreover, changes in the other osseous structures of the TMJ were out of the scope of the present study. Even though degenerative changes in the condyle are more common than articular eminence,[21] their relationship to alterations is still unclear.

Conclusions

Flattening, erosion, and sclerosis were the most common degenerative changes in the articular surface of the condyle. Degenerative condylar changes may affect the dimensions of the TMJ spaces. Increasing age and being male was associated with radiographically detectable degenerative findings in the mandibular condyle. Further longitudinal studies are needed to investigate these findings.

Ethics approval and consent to participate

The local Research Ethics Committee approved the protocol of this multicenter study (No.: 14/3 and Ref.: 36290600/124), and the study complied with the 1964 Declaration of Helsinki ethical guidelines.

Data availability

The datasets generated and/or analyzed during the current study are not publicly available, because they contain information that could compromise the privacy of research participants.

Consent for publication

Not applicable.

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Original paper

Effects of the substructure thickness, the resin cement color and the finishing procedure on the color and translucency of zirconia-based ceramic restorations

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Abstract

Background. The ability to simulate the natural appearance of teeth in dental restorations is one of the most important factors that make treatment successful.

Objectives. The present study evaluated the effects of the substructure thickness, the resin cement color and the finishing procedure on the color and translucency of bilayer zirconia-based ceramic restorations.

Material and methods. Yttrium-stabilized tetragonal zirconia polycrystal (Y-TZP) CAD/CAM blocks (dimensions of 6.0 × 5.5 × 0.4 mm, 6.0 × 5.5 × 0.8 mm, and 6.0 × 5.5 × 1.6 mm) veneered with the fluorapatite-containing ceramics were used. For polishing, the surfaces of half of the test specimens were adjusted with a blue-belted diamond porcelain bur and a white polishing rubber, and the other half were glazed. The test specimens were then cemented with 2 different colors of the same self-adhesive resin cement to the resin composite. A spectrophotometer was used to measure the L*, a*, and b* color attributes of the specimens. Additionally, the ΔE values were calculated to determine color differences between each group and the control. Data was analyzed using the multifactorial repeated-measures analysis of variance (ANOVA) and subgroup analysis ($p < 0.005$).

Results. It was found that the highest substructure thickness resulted in the lowest color change ($\Delta E = 1.24$) ($p < 0.005$). However, a substructure thickness of 0.8 mm showed less color change ($\Delta E = 1.39$) than the 0.4-mm thickness ($\Delta E = 3.85$) in the translucent resin cement/polished subgroup, as measured against a gray background ($p = 0.001$).

Conclusions. The most significant factor in masking the abutment color in zirconia-based restorations is the thickness of the substructure. The surface finishing procedure or the resin cement color do not have a primary effect on the color change or translucency.

Keywords: zirconia, color, surface finishing, resin cements, substructure thickness

Introduction

Metal-supported porcelain restorations show long-term success, given their good mechanical properties.[1] However, from an aesthetic point of view, metal infrastructures have some negative aspects, such as preventing light transmission[2] and metallic color reflection in the gingival region.[3,4] For this reason, metal-supported porcelain restorations, which have been accepted as the gold standard in dentistry for many years, are now being replaced with more aesthetic alternatives, such as zirconia-supported porcelain restorations.[1,5] In these restorations, the zirconia infrastructure provides high strength, while the veneer porcelain helps to achieve a natural appearance.[6]

The translucency of a ceramic material influences the natural appearance of the restoration and affects the esthetics of a restoration. A 1-mm thick zirconia ceramic substructure material has a visible light transmission between 20% and 50%. Therefore, zirconia is defined as a semi-translucent material.[7,8] On the other hand, in cases where discolored teeth, metallic core materials, and titanium abutments need to be masked, the light transmittance of zirconia appears to be a disadvantage rather than an advantage.[6,9] Under these conditions, zirconia cannot mask the dark color, and the final color of the restoration is affected by the substructure color.[9,10] Therefore, the thickness of the veneer and core materials affect the final appearance of ceramic restorations.[11] Wang et al. reported that an increase in zirconia thickness reduces the translucency of the restoration after comparing the light transmittance of dental ceramics with different thicknesses.[12] Similarly, Tabatabaian et al. investigated the effect of zirconia substructure thickness and background type on masking ability and reported that zirconia substructures should be at least 0.4 mm thick to obtain ideal coverage in masking A3.5 colored composite resins.[9] In a study by Tabatabaian et al., the masking ability of zirconia of various thicknesses on white and black backgrounds was evaluated.[6] It was found that 1-mm thick zirconia had the acceptable masking ability, but the required thickness for ideal masking should be 1.6 mm.

In addition to material thickness, the application of glaze porcelain and the cement used can also affect the optical properties of zirconia-supported restorations.[3] The final smooth finish of porcelain surfaces is provided by the application of glaze. Although it is generally accepted that glazed porcelain provides optimal surface quality, it is sometimes necessary for occlusal adjustments during the adaptation of the restoration. In these cases, repeated firing of the restoration will not be practical, so the refinishing of porcelain surfaces on which surface adjustments have been made is done intraorally.[12] Studies have evaluated the color stability of dental ceramics, concluding that accurate finishing procedures could provide surface texture similar to a glazed surface. Therefore, intraoral polishing procedures as an alternative to glazing have also been suggested.[13,14]

Regarding the role of cements on color, a study by Fazi et al. evaluated the effects of four different cements on the color of zirconia-supported ceramics, concluding that opaque cements should be avoided in areas where porcelain thickness decreases in restoration.[15] Although cements have significant effects on the final color of all-ceramic restorations, their effects on zirconia-based restorations are not yet fully known.[3] Since spectrophotometers and colorimeters can show fewer color differences than the human eye can distinguish, detectable and acceptable clinical ΔE threshold values have been defined.[7] The ΔE value obtained is compared with these threshold values to evaluate the visibility of the color difference. Threshold ΔE values between 1 and 5.5 have been reported in the studies. Although these color differences cannot be perceived by the clinician, a ΔE value of less than 2.6 indicates ideal color difference, while a value of less than 5.5 indicates acceptable color changes.[16,17]

Although zirconia-based restorations have aesthetic advantages compared with metal-ceramic restorations, it is still complex to achieve a natural appearance with zirconia-based restorations due to different factors, such as the cement used, zirconia coping, veneering ceramic, laboratory procedure, and surface finishing procedure.[7] This study simulated three variables of a bilayer zirconia ceramic restoration, such as veneering ceramic and core and luting cement. Substructure thickness, color of resin cement, and surface finishing procedure were the parameters used in the evaluation of color change and translucency.

This study aimed to analyze the effect of (i) self-adhesive resin cements with different colors and opacity, (ii) semi-opaque zirconia substructure stabilized with yttrium in different thicknesses, and (iii) glaze porcelain application and mechanical polishing processes on color and light transmission of bilayer zirconia-supported ceramic restorations. The null hypothesis of this study was that the examined material thickness, different polishing protocols, and resin cement color/opacity would affect both color and translucency of zirconia-based ceramic restorations.

Material and methods

Study design

This in vitro study aimed to test the color difference between bilayer Yttrium-stabilized tetragonal zirconia polycrystal (Y-TZP) CAD/CAM blocks with different material configurations of thickness, finishing protocols, and resin cements with different colors and opacity. A sample size of $n = 12$ was used for each group (Fig. 1). The material configuration groups were as follows: color and opacity of resin cement (universal A2 and transparent), finishing protocol (mechanical grinding and glaze), and substructure thickness (0.4 mm, 0.8 mm, and 1.6 mm).

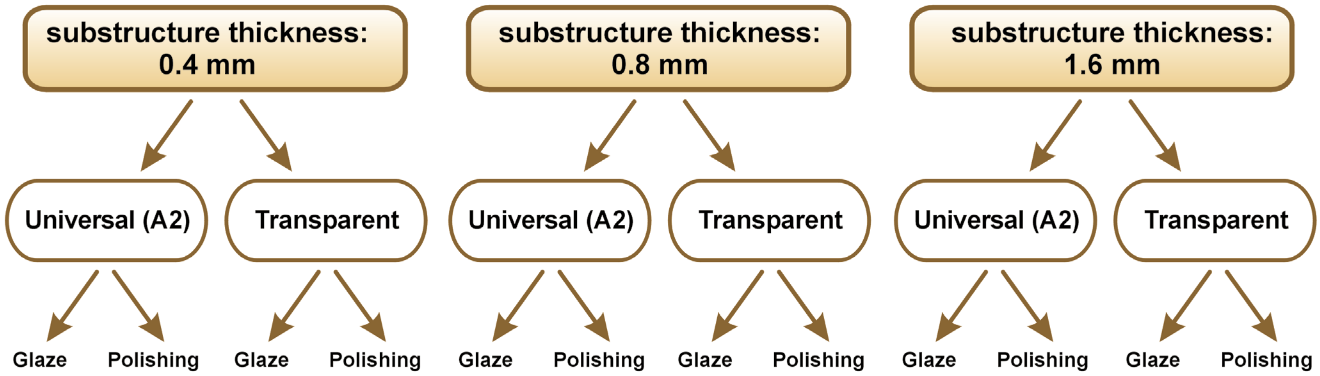

Fig. 1. Study plan and subgroups

Preparation of the yttrium-stabilized zirconia substructure test specimens

The Y-TZP CAD/CAM blocks (IPS e.max ZirCAD, MO 2/C 15; Ivoclar Vivadent AG, Schaan, Liechtenstein) were cut using a precision cutting device (MICRACUT 201; Metkon Instruments Inc., Bursa, Turkey) and a diamond cutting disc (15LC, 11-4255, 127 × 0.4 mm; IsoMet Diamond Wafering Blades, Buehler, USA) (dimensions of 7.5 × 6.9 × 0.5 mm, 7.5 × 6.9 × 1 mm and 7.5 × 6.9 × 2 mm) simulating the substructure of bilayer restorations. The surfaces of the test specimens were polished with 800 and 1200 grit SiC paper (English Abrasives & Chemicals Ltd., Stafford, UK) under running water by the same researcher for 10 s with each SiC paper. Sample thicknesses were checked using a digital caliper (Digitaler Messscheiber; Alpha Tools, Bahag AG, Mannheim, Germany). Before sintering, the samples were cleaned with pressurized steam. All of the test specimens were sintered in a sintering furnace (MoS-B 160/2, Protherm Furnaces; Alser Teknik Seramik Inc., Ankara, Turkey) for 7 h and 20 min at 1,500°C, according to the manufacturer's recommendations. Final test specimen dimensions were measured as 6.0 × 5.5 × 0.4 mm, 6.0 × 5.5 × 0.8 mm, and 6.0 × 5.5 × 1.6 mm, with 20% shrinkage resulting from the sintering process. Sintered zirconia test samples were cleaned with 96% ethanol.

Application of the fluorapatite-containing veneer ceramic to the zirconia specimens

Fluorapatite layering ceramic powder (IPS e.max Ceram Dentin A2/TI1; Ivoclar Vivadent AG) and liquid (IPS e.max Ceram Allround Build Up Liquid; Ivoclar Vivadent AG) were mixed and applied to sintered yttrium-stabilized zirconia test specimens. It was applied by the manual layering technique into aluminum molds with dimensions of 6.5 × 6.5 × 1.4 mm, 6.5 × 6.5 × 1.8 mm, and 6.5 × 6.5 × 2.6 mm and fired in a porcelain furnace (Programat P310; Ivoclar Vivadent AG) according to the instructions. After firing, layering porcelain was added to the missing areas in the mold due to shrinkage (of around 20%) in the porcelain and then fired again. The surfaces of the ceramic samples were adjusted with a blue-belted diamond porcelain bur (Sigmadent, Istanbul, Turkey) and white polishing rubber (Nais Dental Polishers, Sofia, Bulgaria) to standardize the surfaces of the specimens; the procedure was performed for 10 s under water cooling by the same practitioner in a circular motion (rather than in one direction) until the desired dimensions were obtained.

Application of glaze to the test specimens

Glaze paste (IPS Ivocolor Glaze Paste; Ivoclar Vivadent AG) and liquid (IPS Ivocolor Allround Mixing Liquid; Ivoclar Vivadent AG) were applied to half of the test specimens using a brush and fired in the same porcelain furnace according to the manufacturer's instructions.

Preparation of the resin composite test specimens

To imitate tooth color, a resin composite (Charisma Smart A3; Heraeus Kulzer, Hanau, Germany) was placed in a 6.0 × 6.5 × 10 mm plexiglass mold (Ostim, Ankara, Turkey) by the application of a layering technique and polymerized for 20 s, according to the manufacturer's instructions, with a light device (LED.F; Woodpecker Medical Instrument Co., Guilin, China).

Cementation of the ceramic test specimens to the resin composite test specimens

The surfaces of the zirconia test specimens veneered with layering porcelain were sandblasted with 50-µm aluminum oxide particles for 10 s under 60 psi at a distance of 0.5 mm in a sandblaster (Heraeus Kulzer Combilabor, CL FSG 3; Heraeus Kulzer). All specimens were cleaned ultrasonically in distilled water for 10 min. The zirconia ceramic test specimens were cemented to the resin composite test specimens in accordance with the manufacturer's instructions, using two different colors of the same self-adhesive resin cement (Panavia SA Cement Plus Universal [A2] and Transparent, Kuraray Noritake Dental Inc., Okayama, Japan). To standardize the cement thicknesses, 0.13 mm thick perforated teflon fabric (Haksan Industry Materials, Ankara, Turkey) was used. During cementation, the specimens were placed on a metal weight mechanism (Ostim, Ankara, Turkey), and 9.4 kg of force was applied to them throughout the polymerization period.

Color measurements

Color measurements were made before and after the cementation of all ceramic test specimens to the resin composites. A spectrophotometer device (CR-321 Chroma Meter, Konica Minolta, Tokyo, Japan) was used for color measurements with black, gray, and white backgrounds. For the evaluation of color measurement, the CIE L*a*b* color system, which was standardized by the International Commission on Illumination, named Commission internationale de l'éclairage, was used. The CIE L*a*b* system defines color in 3 axes: L*; a*; and b*. The L* axis indicates the degree of lightness, darkness, brightness, or black/white – pure white is 100 L*, while pure black is 0 L*. The a* and b* axes represent the chromatic properties of the color. The a* value indicates the red-green ratio of the color and the b* value indicates the yellow-blue ratio. If the a* value is positive, it is red; if it is negative, it is green. If the b* value is positive, it represents yellow; and if it is negative, it represents blue. The color difference is defined by ΔE. In the calculation of ΔE, the following formula is used (Equation 1):

$$\Delta E_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

If the color change value was $\Delta E \leq 3.7$, it was clinically acceptable.[18,19]

Light transmittance

A spectrophotometer (CR-321 Chroma Meter; Konica Minolta, Tokyo, Japan) was used to measure the light transmittance of the test specimens. Light transmittance was measured using white and black backgrounds. The light transmittance values were calculated using the following formula (Equation 2):

$$TP = \sqrt{\left(L^*_B - L^*_W\right)^2 + \left(a^*_B - a^*_W\right)^2 + \left(b^*_B - b^*_W\right)^2} \quad (2)$$

where:
TP – translucency parameter;
W – color coordinates against a white background; and
B – coordinates against a black background.

Statistical analysis

Post-hoc power analysis was performed with the ClinCalc online post-hoc power calculator. This calculator uses various equations to calculate the statistical power of a study after the study is performed. The study had a power of 86.5–99.9% to produce a significant difference with $N = 24$ participants in terms of ΔE universal A2 (0.4 mm) black background and ΔE translucent (0.8 mm) gray background with 0.05 type 1 error.

All analyses were performed using IBM SPSS Statistics for Windows, v. 24.0 (IBM Corp., Armonk, USA). The conformity of continuous variables to a normal distribution was tested with the Shapiro–Wilk test. Descriptive statistics were used to describe continuous variables: mean (*M*); standard deviation (*SD*); minimum (min); median (*Me*); and maximum (max). The Mann–Whitney *U* test was used to compare two independent and non-normally distributed variables.

The Wilcoxon test was used to compare dependent and non-normally distributed variables. The Friedman test was used when more than two variables did not fit the dependent and normal distribution. Statistical significance was set at a *p*-value of 0.05.

Results

The color of resin cement (A2 or translucent) used did not make a sigignificant difference in terms of the final color of the restoration with different finishing procedures and substructure thicknesses ($p > 0.05$), except for 2 subgroups (Tables 1–4).

The ΔE was statistically significantly higher for the universal A2 resin cement than translucent resin cement in the 0.8 mm polishing gray background group ($p = 0.009$) (Table 2). In contrast, ΔE was significantly higher for translucent resin cement than for universal A2 resin cement in the 0.4 mm glaze white background group ($p = 0.015$) (Table 3).

Polishing procedure did not have a significant effect on the color of the test specimens with a white background (Table 3). Conversely, polishing significantly increased the color change as compared with glaze in the 0.4 mm substructure thickness subgroup of universal A2 resin cement with a black background and translucent resin cement with a gray background ($p = 0.002$ and $p = 0.028$, respectively). In addition, a statistically significant higher color change was achieved with glaze as compared with polishing in the 0.8 mm substructure thickness subgroup of translucent resin cement with a gray background ($p = 0.041$).

The results of our study also indicated that there was a statistically significant change in the color of the tested specimens due to substructure thickness ($p > 0.05$). For both universal A2 and translucent resin cement, a lower color change was observed in the 1.6 mm substructure thickness subgroup compared with 0.4 mm and 0.8 mm thicknesses with black backgrounds ($p < 0.05$). Polishing of the 1.6 mm substructure in the universal A2 resin cement subgroup and glaze in the translucent resin cement subgroup resulted in a lower color change than 0.4 mm substructure thickness with both gray and white backgrounds ($p < 0.05$). Additionally, the 0.8 mm substructure thickness had a greater color change than the 0.4 mm in the subgroup, which was cemented with a translucent resin cement/polished subgroup and measured with a gray background ($p = 0.001$).

Table 1. Multiple comparison of the mean color change (ΔE) values among different substructure thickness, resin cement color and surface finishing procedure subgroups (black background)

Parameter	Resin cement color	Surface finishing procedure	Substructure thickness			*p*-value
			0.4 mm	0.8 mm	1.6 mm	
ΔE	universal A2	polishing	3.8[b]	3.62[a]	1.24[ab]	<0.001*
		glaze	2.02[d]	3.99[cd]	2.07[c]	0.001*
	p-value	–	0.002*	0.388	0.099	–
	translucent	polishing	3.77[e]	3.94[f]	1.61[ef]	0.001*
		glaze	2.49[g]	4.14[gh]	2.21[h]	0.001*
	p-value	–	0.099	0.754	0.071	–

* statistically significant; the letters in superscript indicate statistically significant differences among the resin cement color and the substructure thickness.

Table 2. Multiple comparison of the mean color change (ΔE) values among different substructure thickness, resin cement color and surface finishing procedure subgroups (gray background)

Parameter	Resin cement color	Surface finishing procedure	Substructure thickness			*p*-value
			0.4 mm	0.8 mm	1.6 mm	
ΔE	universal A2	polishing	4.22[ab]	2.15[bf]	2.34[a]	0.017*
		glaze	2.57	1.62	2.48	0.205
	p-value	–	0.050	0.239	0.695	–
	translucent	polishing	3.85[c]	1.39[cf]	2.1	0.001*
		glaze	3.12[d]	2.29[e]	2.04[de]	0.017*
	p-value	–	0.028*	0.041*	0.937	–

* statistically significant; the letters in superscript indicate statistically significant differences among the resin cement color and the substructure thickness.

Table 3. Multiple comparison of the mean color change (ΔE) values among different substructure thickness, resin cement color and surface finishing procedure subgroups (white background)

Parameter	Resin cement color	Surface finishing procedure	Substructure thickness			*p*-value
			0.4 mm	0.8 mm	1.6 mm	
ΔE	universal A2	polishing	2.78[a]	1.97	1.45[a]	0.039*
		glaze	2.09[b]	1.53	1.8	0.779
	p-value	–	0.099	0.239	0.158	–
	translucent	polishing	2.25	2.25	1.32	0.125
		glaze	2.82[b]	1.55[b]	2.23[b]	0.001*
	p-value	–	0.754	0.136	0.050	–

* statistically significant; the letters in superscript indicate statistically significant differences among the resin cement color and the substructure thickness.

Table 4. Multiple comparison of the mean translucency parameter (TP) values among different substructure thickness, resin cement color and surface finishing procedure subgroups

Parameter	Resin cement color	Surface finishing procedure	Substructure thickness			*p*-value
			0.4 mm	0.8 mm	1.6 mm	
TP	universal A2	polishing	0.84	0.77	1.08	0.338
		glaze	0.71	0.92	0.87	0.205
	p-value	–	0.433	0.433	0.338	–
	translucent	polishing	0.70	0.77	0.98	0.558
		glaze	1.24	1.13	0.91	0.779
	p-value	–	0.060	0.136	0.694	–

The highest translucency value was observed in a test group with a glazed 0.4 mm substructure cemented with translucent resin cement, and the lowest was a polished one. However, substructure thickness, resin cement color, and surface finishing procedure had no statistically significant effect on the translucency parameter of test specimens ($p > 0.05$) (Table 4).

Discussion

For all parameters, the null hypothesis was accepted. A 1.6 mm substructure ceramic thickness met the clinically acceptable color change when considering the three analyzed backgrounds. Reducing the substructure thickness to 0.4 or 0.8 mm caused a greater color change than the clinically acceptable value, especially in subgroups with polishing and gray backgrounds (Table 2). However, 0.4 mm and 0.8 mm substructure thicknesses and glazed test specimens generally resulted in clinically acceptable color change values with each background (Tables 1–3). These results indicate that when clinically low substructure thickness is required, it will be clinically more appropriate to use glaze as a surface finishing process.

Translucent ceramic restorations should be restricted to abutments that are closely similar to the planned final color of the restorations. However, in the presence of clinically colored tooth tissue, it may be necessary to use opaque materials, such as zirconia ceramics, that can camouflage the color of abutment. However, in this case, the substructure thickness of zirconia ceramics can be decisive in the final color of the restoration. Previous studies have shown that the color of restorations is significantly affected by ceramic thickness.[11] Similarly, we found substructure thickness to be the most significant variable in color change, especially with black background (Table 1). Hence, the thickness was concluded to be a major determinant in achieving ideal clinical standards in this in vitro study. For both colors of resin cement, a lower color change was observed in the subgroup of 1.6 mm substructure thickness compared with 0.4 mm and 0.8 mm with a black background ($p < 0.05$). As expected, the highest values of ΔE were found with thin substructure thicknesses (0.4 mm). Additionally, 1.6 mm substructure thickness with polishing in the universal A2 resin cement subgroup and glazing in the translucent resin cement subgroup resulted in a lower color change than 0.4 mm substructure thickness with both gray and white backgrounds ($p < 0.05$). Taken together, these results indicate that a higher substructure thickness can better camouflage the abutment color.

Similar to our study, Tabatabaian et al. reported that masking ability increased as the zirconia ceramic thickness increased, with ideal masking ability at a minimum thickness of 1.6 mm.[9] In contrast, in studies by Fazi et al.[15] and Sinmazisik et al.,[20] core thickness surprisingly had no significant effect on ΔE. The reason for this may be related to the fact that the core thicknesses tested in the study by Fazi et al.[15] were less than the substructure thicknesses tested in our study. Further studies should support our data and reveal the minimal core thickness to minimize tooth background interferences on the final color of the restoration.

Generally, it has been reported that polishing surfaces after crown recontouring or occlusal adjustments should be avoided and reglazed before definitive cementation.[21,22] However, various polishing procedures could be used on ceramic surfaces to obtain structural resistance and a clinically acceptable smoothness compared with glazing.[22] In addition, surface texture influences the color of ceramic restorations. Thus, one of the aims of our study was to compare ΔE values before and after cementation among subgroups with surface finishing procedures. Our results did not indicate perceptible color changes between polishing versus glaze with white backgrounds across the tested ceramics (Table 3). These results were similar to those of a study by Akar et al.[19] The ΔE values after each surface treatment method may be affected by the opaque structure of the zirconia. Conversely, polishing increased color change compared with glaze in the 0.4 mm substructure thickness subgroup of Universal A2 resin cement with a black background and translucent resin cement with a gray background ($p = 0.002$ and $p = 0.028$, respectively). In addition, a higher color change occurred with glaze compared with polishing in the 0.8 mm substructure thickness subgroup of translucent resin cement with a gray background ($p = 0.041$). The difference between these groups was thought to be related to the background color used. On the other hand, ΔE values were above clinically acceptable values in groups with polishing and a 0.4 mm substructure thickness (Table 2). This suggests that the color camouflage effectiveness of the polishing process is low compared with glaze, especially with a low substructure thickness. In contrast, no significant differences were found between glazed and polished based on clinically acceptable color changes ($p < 0.05$), similar to a study by Aldosari et al. that evaluated surface roughness and color measurements of glazed or polished hybrid, feldspathic, and Zirconia CAD/CAM restorative materials after hot and cold coffee immersion; however, the values were within clinically acceptable values ($\Delta E \leq 3.7$).[22]

Both tested resin cements presented limited variations in ΔE when compared with one another. ΔE was higher for Universal A2 resin cement than translucent resin cement in the 0.8 mm polishing gray background group ($p = 0.009$) (Table 2). In contrast, E was higher for translucent resin cement than Universal A2 resin cement in the 0.4 mm glaze white background group ($p = 0.015$) (Table 3). This result shows that as the substrate thickness decreased, the translucent nature of the resin cement used changed the final restoration color more after cementation; that is, it was more affected by the substructure color in this test group. In general, similar to the study by

Fazi et al., most of the recorded ΔE values were below the limit of clinical acceptance and not statistically significant in terms of the color of cement used ($p > 0.005$), except for two subgroups. This suggests that choosing a cement shade of the same color as the ceramic (A2) or translucent might not affect the final ideal color of restorations.

Wang et al. reported that a significant increase in translucency was found as a result of a decrease in ceramic thickness. The TP value of human dentin with a thickness of 1.0 mm has been determined to be 16.4 and that of human enamel to be 18.1.[23] In our study of bilayer zirconia ceramics, the TP, which ranged from 0.7 to 1.24, was less than that for human dentin and enamel. However, the results in Table 4 indicate that there are no statistically significant differences in the translucency of the tested specimens in terms of surface finishing procedure, resin cement color, or substructure thickness ($p > 0.05$). The highest translucency value was observed in the glazed 0.4 mm substructure test group cemented with translucent resin cement (1.24), and the lowest was in the polished one (0.7). Similar to our study, Tabatabaian et al.[9] and Wang et al.[23] reported that an increase in the material thickness decreased the TP. However, these differences were not statistically significant in our study. These differences could be related to the selected brand of zirconia or the difference in thickness of the tested substructure.

Although clinical studies are needed to confirm these findings, the results of our study suggest that significant color changes may occur after the cementation of a bilayer zirconia restoration, particularly when the thickness of the substructure is limited. This study provides additional evidence supporting the use of bilayer CAD/CAM zirconia ceramics to help overcome the clinical challenge of aesthetically masking dark abutments, such as metal abutments and discolored tooth substrates.

Limitations

This study had some limitations, such as the use of a specific brand of zirconia ceramic and resin cement. Additionally, the use of resin composite to imitate tooth color may not replicate the optical effects of natural teeth. Further investigation of these factors is needed.

Conclusions

As the substructure thickness increased, the effect of resin cement and surface finishing procedure on the resultant restoration color decreased. Overall, no significant difference was observed between glaze versus polishing for 0.8- and 1.6-mm substructure thicknesses in the final restoration color. Glaze should be preferred over polishing to achieve camouflage effectiveness in clinical situations where the substructure thickness is 0.4 mm. The color of the resin cement used did not have a significant effect on the final color of the bilayer zirconia restoration, regardless of the preferred substructure thickness and surface finishing procedure. The thickness of the substructure, surface finishing procedure, or color of resin cement did not affect the translucency of the tested bilayer zirconia restorations.

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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Review

Prevalence of periodontal disease among Vietnamese adults: A systematic review and meta-analysis

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

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Abstract

To synthesize previous findings on the prevalence of periodontal disease (PD) in the adult Vietnamese population, a search for peer-reviewed literature was conducted using the MEDLINE, PubMed and Scopus databases through January 10, 2022. Two reviewers individually assessed abstracts and full-text articles to determine their suitability for inclusion. Only English articles were included if their results described the prevalence of PD among the Vietnamese. Among 900 potential studies, 8 cross-sectional studies with 7,262 adult participants qualified to be included. We found that overall the prevalence of PD was 64.9% (95% confidence interval (*CI*): 45–81%), with very high heterogeneity across the observed prevalence estimates ($Q = 1,204.8776$; $df = 7$; $p < 0.001$; $I^2 = 99.42\%$). Further subgroup analyses stratified by age, location, sampling, study design, and region also revealed significant differences, with a higher prevalence of PD among (1) population-based studies, (2) participants aged ≥65 years, (3) participants with non-chronic diseases, (4) studies using the WHO, community periodontal index (CPI) and standard oral examinations, (5) studies conducted in Central Vietnam, and (6) studies using randomization sampling ($p < 0.01$) than in other populations. Sensitivity analyses validated the stability of the current findings. Within the limits of the available evidence, this meta-analysis showed a high percentage of Vietnamese adults suffer from PD. Nonetheless, the findings should be taken cautiously due to the limited number of published articles and the possibility of bias in the included research. More well-designed studies with larger sample sizes are thus required for further verification.

Keywords: rheumatoid arthritis, inflammation, periodontitis

Cite as
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Introduction

Maintaining excellent oral health is not just about keeping our teeth healthy, but it is also about maintaining our overall health and well-being, as has been extensively proven in the literature. Thus, the World Health Organization (WHO) expanded the definition of oral health by including the concept of well-being as "Oral health is a key indicator of overall health, well-being, and quality of life".[1] Gingivitis- and periodontitis-induced tissue deterioration are included under the umbrella term of periodontal disease (PD), which results in alveolar bone and tooth loss. Masticatory dysfunction and an adverse effect on the patient's quality of life have been linked to PD.[2] Even though oral diseases are generally avoidable, they are ubiquitous throughout life and have significant negative consequences for the health of individuals, groups, and society at large, with substantial social, psychological, health services, and economic impacts.[3] A serious public health concern, periodontitis significantly raises morbidity and expenditures on dental healthcare, affecting around 14% of the world's adult population in 2020.[1] Although PD substantially influences one's overall health and well-being, this disease has received just a fraction of the attention of dental caries in terms of public health approaches to disease management and prevention.

Unfortunately, many nations still lack the human, financial, and material resources necessary to fulfill the demand for oral health care services and a comprehensive program to provide universal access, particularly in developing countries such as Vietnam.[4] The 2001 National Oral Health Survey showed the prevalence of PD amongst adults is exceedingly high. According to this survey, PD affected 97.5% of Vietnamese, and a staggering 91.8% of Vietnamese adults 45 years and older had gingivitis and PD.[5] Over the last several decades, Vietnam has made significant strides toward improving public health. As a result of the significant burden of PD and its consequences for individuals and healthcare systems, the Vietnamese government has made a considerable effort to improve the oral health of its citizens; however, the results have fallen short of expectations.[6] Moreover, a comprehensive public health strategy to prevent PD and enhance oral health is now in the early stages of development.

To the best of our knowledge, no systematic review studies regarding PD in Vietnamese adults have been conducted. Therefore, it is critical to raise awareness of PD and understand the state of knowledge on the condition as a significant component of reorienting health services to improve periodontal health. For these reasons, we conducted a systematic review to critically and comprehensively determine the percentage of individuals in the Vietnamese community who have PD.

Material and methods

Protocol

An effective search and selection strategy was developed according to the principles and suggestions laid forth in the PRISMA Statement[7] to guarantee that the review process was transparent and exhaustive.

Search strategy

We used a predetermined lexicon to conduct a comprehensive search up to January 10, 2022. In three databases comprising a gray literature database (MEDLINE, PubMed and Scopus), the language was confined to English. Consequently, there is a possibility for publishing bias to be introduced. Depending on the database, we used a combination of keywords, medical subject headings (MeSH), synonyms, and glossary terms to enhance our search results. Search terms included oral diseases (e.g., periodontitis, tooth loss, mouth cancer) and the relevant population (e.g., Vietnam, Vietnamese). Appendix 1 (available on request from the corresponding author) shows a synopsis of the search approach.

Inclusion and exclusion criteria

The studies were screened based on titles and abstracts. If an informed conclusion could not be reached based on this information, the entire article was examined. Any English-language observational research (such as a cohort, case-control, or cross-sectional study) was eligible if it met all of the following criteria:

- The original research was published in a peer-reviewed journal reporting outcomes on the proportion of PD in Vietnamese patients who reside in the country.
- Only studies in which a dental professional performed an oral/dental examination were included.
- The research included adult participants who were at least 18 years old.

Articles were ineligible if they satisfied any of the following criteria:

- Duplications were eliminated from the evaluation process.
- Experimental studies, as well as animal studies and editorial letters, reviews, commentaries, book chapters, and case studies, were excluded.
- Participants were an overseas Vietnamese population living outside Vietnam.
- Neither the whole text nor any relevant information could be retrieved.

There was no screening applied to the recruiting sites (e.g., care homes, hospitals, or communities) or the general health state of the participants in this study.

Study selection and data extraction

According to the PRISMA flow diagram, the selection process was carried out step-by-step (Fig. 1). The following screening procedures were completed by two separate reviewers evaluating the original collection of articles (CTQV and QNP):

– First, duplications and publications written in languages other than English were ruled out.
– Second, the description in the abstracts was used to make further exclusions. Discarded articles were those that did not meet the criteria for inclusion. Full-text screenings were conducted where the information for inclusion and/or exclusion was not included in the abstract.
– Finally, the full texts of the remaining articles were scrutinized to avoid biased inclusion of studies, and those that did not meet the qualifying requirements were once again discarded.

Any disagreements between authors about whether a study was eligible were discussed and adjudicated by the third reviewer (DQT). Microsoft Excel 2019 was used to keep track of all of the chosen references for retrieval from the databases. Table 1 contains the information gathered from the data retrieval, which includes the first author, publication year, study design, geographical location (community or hospital), sample size, average age, gender, type of oral assessment, and population type (non-patient and patient participants).

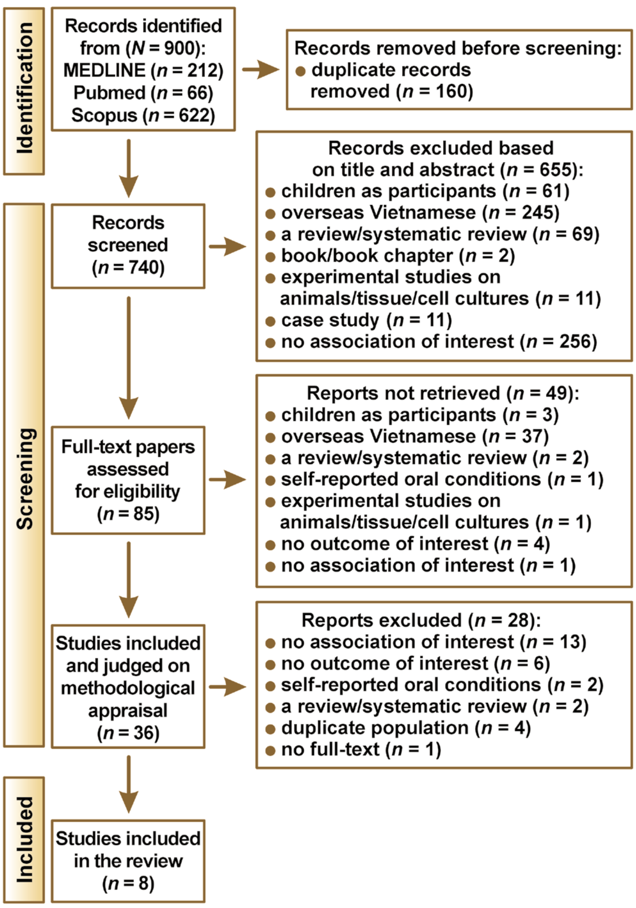

Fig. 1. PRISMA flowchart depicting the process of systematic review inclusion and exclusion

Quality assessment

PNQ and CTQV independently evaluated the methodological quality of the included papers using the Newcastle–Ottawa Scale for cross-sectional studies. This scale evaluates three domains, including the representativeness of the investigated subjects, the comparability across groups, and the ascertainment of outcome and exposure. The overall score for a cross-sectional study was 8 points. The included studies were then divided into three categories: high (>7 points), moderate (4–6 points), and poor (0–3 points) in terms of overall quality scores.[8] Any dispute between the two reviewers about the quality of methodology and evidence score of the included articles was adjudicated by DQT.

Statistical analysis

The primary finding of this study was the prevalence of PD among the Vietnamese adult population. For all analyses, we utilized RStudio Desktop, v. 2022.02.0 (https://posit.co/download/rstudio-desktop), together with the meta and metafor packages. Heterogeneity was assessed by Cochran's Q-statistic and I^2 tests. A fixed-effects model was used if there was no substantial heterogeneity identified ($I^2 \leq 50\%$; $p > 0.1$); otherwise, a random-effects model was adopted. Sensitivity analyses were performed to determine the robustness of the overall estimates. We undertook subgroup analysis by areas, assessment, average age, regional differences, sampling, and non-patient and patient participants. Subgroup comparisons were statistically significant if the significance threshold was a *p*-value ≤0.05. To evaluate publication bias, funnel plots were used.

Results

Results of the search

A total of 900 potentially relevant articles were identified from all of the databases used in this study. Following the removal of duplicates, 740 results were analyzed further. Titles and abstracts were evaluated for research methodology, research site, participant, reporting on oral health status, and the method for evaluating the participants' dental health. Following the screening of titles and abstracts, 85 studies required evaluation of their full text, of which 77 did not fulfill the selection criteria and were thus discarded. Ultimately, we explored eight selected original articles for inclusion in this review. Results of the systematic searches and details of the selection process are shown in Fig. 1 and Appendix 2 (available on request from the corresponding author), respectively.

Table 1. Characteristics of the included studies, percentage of periodontal disease (PD) and pertinent variables

No.	Author/year of publication	Study methodology	Population characteristics	Diagnostic criteria for PD	Quality
1	Nguyen et al. 2018[9]	– cross-sectional study – elderly people ($n = 258$) – in population/Central Vietnam (in Da Nang) – multi-stage stratified random sampling	age: 65–74 years (no mean age provided) 130 males (50.4%) and 128 females (49.6%)	CPI	moderate
2	Pham et al. 2018[11]	– cross-sectional study – elderly ($n = 791$) – at hospital/Southern Vietnam (in 3 nursing homes in Ho Chi Minh)	mean age: 72.9 ±9.1 years 360 males (45.5%) and 431 females (54.5%)	WHO†	moderate
3	Pham et al. 2018[12]	– cross-sectional study – adult patients ($n = 367$) – at hospital/Southern Vietnam (at the Faculty of Odonto-Stomatology, University of Medicine and Pharmacy, Ho Chi Minh) – convenience sample	mean age: 34.9 ±13.5 years 40.3% males and 59.7% females	standard oral examination	moderate
4	Pham 2018[13]	– cross-sectional study – metabolic syndrome patients ($n = 412$) – at hospital/Southern Vietnam (at the Examination Department of the Institute of Traditional Medicine, Ho Chi Minh) – convenience sample	mean age: 57.8 ±5.7 years 114 males and 298 females	CDC/AAP‡	moderate
5	Nguyen et al. 2020[14]	– cross-sectional study – rheumatoid arthritis and osteoarthritis patients ($n = 300$) – at hospital/Southern Vietnam (at the Department of Rheumatology, Cho Ray Hospital, Ho Chi Minh) – convenience sample	age: 27–84 years 18% males and 82% females	CDC/AAP‡	moderate
6	Dao et al. 2015[10]	– cross-sectional study – adult patients ($n = 799$) – at hospital/Central Vietnam (at 3 commune health centers in Thua Thien Hue province) – convenience sample	age: >18 years 23.4% males and 76.6% females	WHO†	high
7	Pham and Tran 2018[15]	– cross-sectional study – obesity and diabetes patients ($n = 679$) – at hospital/Southern Vietnam (at the Institute of Traditional Medicine, Ho Chi Minh) – convenience sample	mean age: 47.9 ±16.7 years 182 males (26.8%) and 497 females (73.2%)	CDC/AAP‡	moderate
8	Do et al. 2011[5]	– National Oral Health Survey – Vietnamese population – in population – multi-stage stratified random sampling	no mean age provided 39.5% males and 60.5% females	WHO†	high

CPI – modified community periodontal index; WHO – World Health Organization; CDC/AAP – Centers for Disease Control and Prevention/the American Academy of Periodontology.
† WHO. Oral Health Survey. Basic Methods. 1997. ‡ Centers for Disease Control and Prevention and the American Academy of Periodontology. 2012.

Findings

Table 1 summarizes the research characteristics of the studies included in the current meta-analysis. A total of 7,262 participants were included in the eight investigations, which comprised one nationwide study[5] and seven cross-sectional studies.[9–15] Two studies were performed in Central Vietnam,[9,10] five studies in Southern Vietnam,[11–15] and one nationwide study in 14 provinces and cities, which in total represents 62 of the provinces over the whole country.[5] All of the research was carried out between 1999 and 2020. Six of these studies were done in clinical departments of a hospital,[10–15] while two utilized a population database to recruit participants.[5,9] Adults of various ages were involved in all studies, and among them, two investigations, exclusively including the elderly (≥60 years), were conducted.[9,11] To diagnose PD, a trained dentist who used a variety of criteria to determine the presence of this disease evaluated the patients, as described in Table 1. Standard criteria were used for the diagnosis and categorized into four groups: Community Periodontal Index (CPI),[9] the Centers for Disease Control and Prevention/the American Academy of Periodontology (CDC/AAP) case definitions for surveillance of periodontitis,[13–15] Oral health surveys: basic methods of WHO,[10,11,16] and a standard oral examination.[12]

Prevalence estimates and heterogeneity

Figure 2 presents the findings of the meta-analysis. The results of a random-effects meta-analysis revealed that the pooled estimate of PD was 64.9% (95% confidence interval (*CI*): 45–81%). Cochran Q was significant (Q = 1,204.8776; df = 7; $p < 0.001$), demonstrating heterogeneity among the prevalence estimations, while the I^2 statistic was 99.42%, indicating very high heterogeneity.

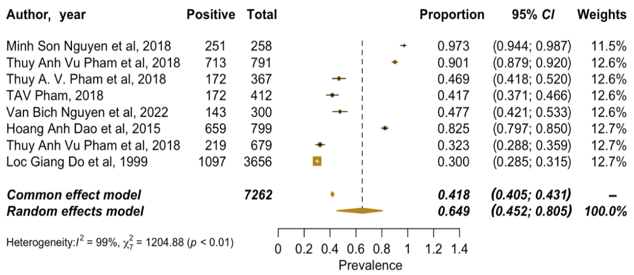

Fig. 2. Forest plots for periodontal disease (PD) in the Vietnamese population during the 1999–2020 period

Subgroup analysis

Areas

In a subgroup analysis of the eight studies of 3,426 periodontal cases, 78.0% (95% *CI*: 37.7–95.4%) were identified in the community, and 60.1% (95% *CI*: 35.6–80.4%) were diagnosed in the hospital. There was high heterogeneity between studies ($p < 0.01$; $I^2 = 99\%$).

Assessment

In the subgroup analysis using assessment tools for PD, 3 studies reported significant differences between the CDC/AAP (40.4%; 95% *CI*: 13.0–75.4%) and the other tools, including WHO, CPI, and standard oral examination (77.5%; 95% *CI*: 51.5–91.8%), with high heterogeneity ($p < 0.01$; $I^2 = 99\%$).

Average age

Over 65-year-old individuals were more likely to develop PD than those under 65 years old (94.5%; 95% *CI*: 82.8–98.4% vs. 47.5%; 95% *CI*: 30.9–64.6%), with high heterogeneity ($p < 0.01$; $I^2 = 99\%$).

Regional differences

The meta-analysis demonstrated that the proportion of PD in Central Vietnam (92.6%; 95% *CI*: 67.9–98.7%) was significantly higher than its proportion in both general (30.0%; 95% *CI*: 29.0–32.0%) and Southern Vietnam (54.5%; 95% *CI*: 28.5–78.3%). There was evidence of high heterogeneity across the studies ($p < 0.01$; $I^2 = 99\%$).

Sampling

The percentage of periodontal cases yielded by randomization sampling was more significant than participants acquired through convenience sampling (78.0%; 95% *CI*: 37.7–95.4% vs. 60.1%; 95% *CI*: 35.6–80.4%), with high heterogeneity ($p < 0.01$; $I^2 = 99\%$).

Non-patient and patient participants

Pooled results from 8 studies indicated that a lower proportion of patients had PD among those with chronic diseases (40.4%; 95% *CI*: 13.0–75.4%) compared with non-chronic diseases (77.5%; 95% *CI*: 51.5–91.8%), with high heterogeneity ($p < 0.01$; $I^2 = 99\%$).

Quality assessment

Table 1 and Appendix 1 (available on request from the corresponding author) depict the study quality appraisal of the included studies. The general quality of the included studies was moderate, with total ratings ranging from five to seven out of a possible eight. The most significant drawbacks of the included studies were selection bias, which occurred since the majority of the studies were not randomized, were done using convenience and purposeful samples, and did not give all of the necessary information for statistical sample size calculation, hence, lacked transparency and completeness.

Publication bias

As shown in Fig. 3, the funnel plot for the primary outcome, PD, appeared somewhat discordant with the results of the meta-analyses. In this case, however, the estimate of publication bias may be erroneous due to a lack of an appropriate number of included studies.

Sensitivity analysis

To determine the stability of the pooled prevalence of our key outcome, each study was eliminated one at a time from the other (Fig. 4). Excluding any one of the studies was shown to not significantly affect the results, indicating that the findings are credible and there is a high prevalence of PD among the Vietnamese population.

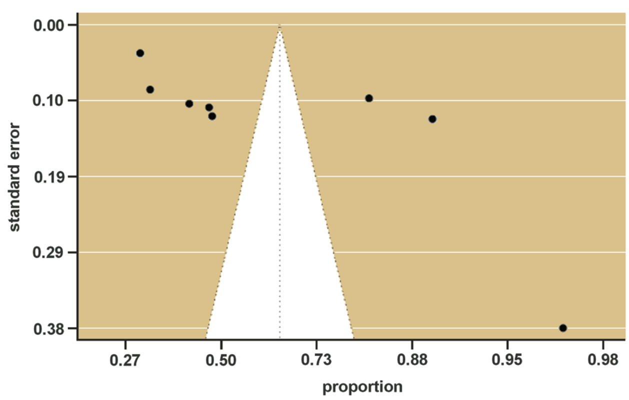

Fig. 3. Forest plot for the meta-analysis of periodontal disease (PD) in the Vietnamese population

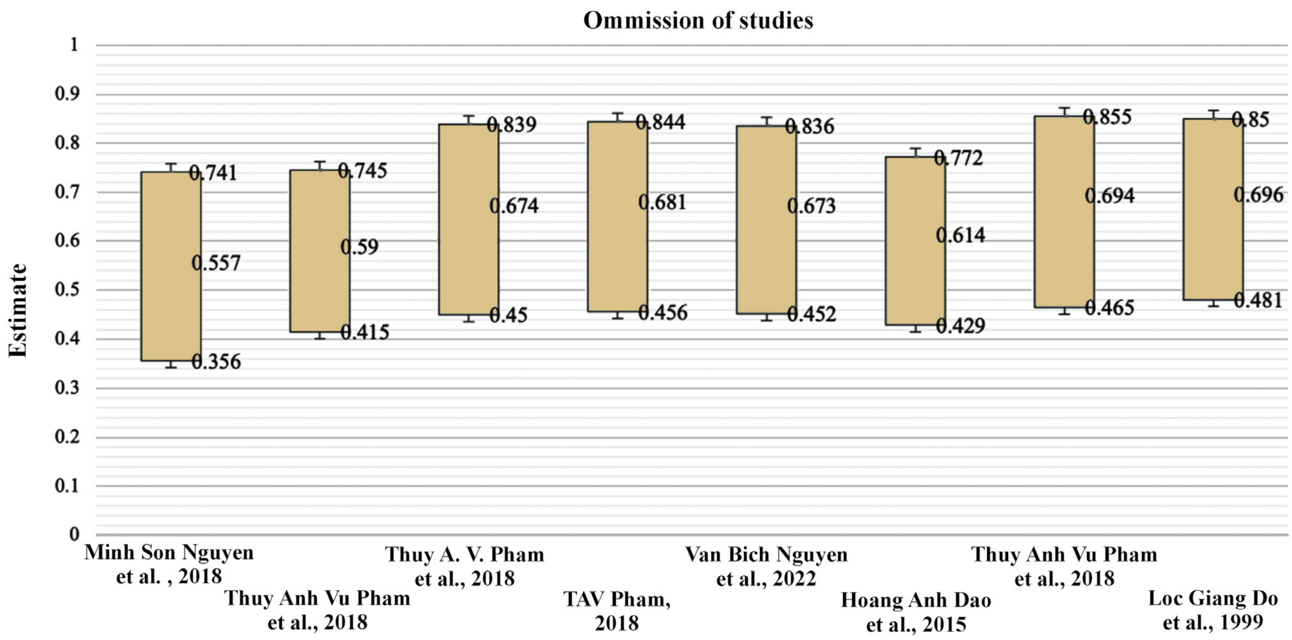

Fig. 4. Sensitivity analysis was subjected to several iterations, each of which excluded one research study while pooling the estimate from the remaining studies

Discussion

According to a WHO report in 2020, more than a billion people are afflicted by PD globally, occurring in both developed and developing nations.[1] Recognizing that PD is avoidable, there is undoubtedly a pressing need for a more global effort to implement effective diagnostic, preventive, and therapeutic strategies to reduce the burden of PD on individuals, nations, and the globe.[17] Therefore, we did this systematic review to synthesize the existing research to assist us in better understanding the prevalence of PD among the Vietnamese adult population.

As a result, a total of 7,262 individuals from eight cross-sectional studies published after the year 1999 were pooled for the first time in this meta-analysis to examine the prevalence of PD in the Vietnamese population. According to the random effect size estimation, the pooled prevalence of PD in this meta-analysis was 64.9%. This estimate was greater than the prevalence of PD in Americans (47.2%),[18] Indian adults (51.0%),[19] and the global prevalence rate of 20–50%.[20] There is a disparity in prevalence estimates between this study and the American[18] and Indian studies,[19] which might be attributed to the differences in methodological quality. Moreover, our results demonstrate that the percentage of PD was considerably lower among individuals who were chronic patients and were receiving treatment at the hospital, studies utilizing the CDC/AAP evaluation and convenience sample, age ≤65 years, and located in Southern Vietnam.

Like many chronic illnesses, PD is a multi-factorial disease with numerous contributing causes, including modifiable and non-modifiable risk factors.[21] From the perspective of public health on a national level, we consolidated several causation hypotheses that may serve as proliferation causes in the development of PD to the expansion of this disease across Vietnam. However, to confirm whether these hypotheses are the genuine causes of PD, they must be explicitly investigated using high consensus criteria for quality research designs and well-validated metrics for the Vietnamese population, which are currently unavailable.

First, among modifiable risk factors for PD, smoking is the most important, and it continues to be a public health concern in many countries, including Vietnam. Several studies have reported that destructive PD is at least five to six times more likely to occur in smokers than in nonsmokers.[22] Despite the Vietnamese government's efforts to eradicate this epidemic, almost half of adult males in Vietnam are now smoking.[23] Moreover, a high number of individuals are exposed to second-hand smoke.[24]

Second, sugar consumption, a foundational element of human development, has been identified as a critical risk factor for the development of PD.[25] Vietnam has been undergoing a nutrition transition, including increasing the availability of sweets and sweetened drinks over the last few years.[26] Moreover, the National Oral Health Survey in 2019 identified numerous notable changes, as remarkable economic development resulted in considerable changes in food consumption, particularly sugar-sweetened foods.[27] The rising consumption of sugar-sweetened foods and beverages among Vietnamese has become a significant public health problem, with daily sugar intake about double the WHO recommendation (25 g).[28]

Third, innumerable research has shown that diabetes mellitus and cardiovascular disease increase the susceptibility to oral infections, especially PD.[21] Among the Vietnamese population, both of these illnesses were significant contributors to the burden of chronic noncommunicable diseases, with the number of persons with diabetes in Vietnam being approximately 5.76 million. In contrast, cardiovascular disease accounted for 31% of all fatalities in 2016.[29]

Forth, PD is one of several ailments whose prevalence increases with an accelerated aging condition. The aging of the world's population is a worldwide phenomenon expected to have profound social transformations in the 21st century[30] as a consequence of a dropping fertility rate and longer life expectancies. Vietnam has one of the oldest populations among Southeast Asian nations.[31] Since PD is a frequent oral health concern among the elderly, our results have gained prominence as a serious public health issue in Vietnam.

Finally, for many people in underdeveloped nations, the expense of treating oral disorders is prohibitive. Thus, they avoid treatment,[32] and people in poor socioeconomic classes are more likely to suffer from dental disease, according to specific epidemiological research.[33] With approximately 97 million people, Vietnam is a lower-middle-income nation.[34] The lack of governmental funding for dental treatment in Vietnam means that many Vietnamese citizens must make tough financial decisions regarding getting dental care. Therefore, economic challenges may have a detrimental impact on the quality of oral health, and poor oral health can have a negative impact on overall health.[35]

Limitations and strengths

In this review, there are certain limitations:

– The majority of the studies were done in a hospital context or healthcare setting, which led to selection bias and over-representation of certain groups. The findings are difficult to extrapolate to the prevalence of PD among Vietnamese communities.
– All the research included in this meta-analysis used a cross-sectional study design, which insufficiently demonstrates ability, and hence, no causal conclusions could be drawn.
– The combined indicators were not comprehensive because of the small number of included papers, which resulted in significant heterogeneity among studies, and potential bias might exist. Therefore, an additional investigation was required.
– We discovered high heterogeneity among studies, which may be due to differences in methodological features, such as various ways of assessing PD.
– There is a lack of consistency in study designs, the definitions of PD, procedures used for disease detection and measurement, and criteria used for subject selection, which substantially restricts the interpretation and analysis of the PD data from the included studies.
– We only included peer-reviewed English-language articles, which may have resulted in the omission of several pertinent research studies, such as the National Oral Health Survey of Vietnam 2001 and 2019.

Accordingly, given the limitations discussed above, the outcomes of this meta-analysis must be confirmed by further well-designed studies from multi-centers with a larger sample size before they can be considered conclusive.

Compared to single-study approaches, our findings have aggregated the most up-to-date evidence to provide more reliable estimates on the percentage of adult patients in Vietnam who have PD. This was achievable since our outcome measure was more standardized, thanks to dental and medical specialists. Our meta-analysis also draws attention to the dearth of literature regarding factors that may contribute to PD in Vietnamese adults. Despite the comprehensive nature of our search technique, we did not summarize the relevant factors that emerged from the included articles. Therefore, future meta-analysis studies should thus focus on the risk factors for PD in adults, particularly those with long-term health conditions. This will help assess the efficiency of PD prevention and treatment actions that use existing risk approaches to minimize the scale of PD and other chronic diseases. Furthermore, raising awareness about PD is a significant public health concern in Vietnam for non-communicable diseases.

Conclusions

The recent pooled analysis of prevalence studies demonstrates that adults with PD are common in Vietnam. Nonetheless, our findings should be taken cautiously due to the small number of publications and the possibility of bias in the included studies. Thus, we urge that further research with rigorous assessments of the prevalence of PD be conducted to validate our results.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets and all supplementary materials 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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Review

Treatment outcome for dentin hypersensitivity with laser therapy: Systematic review and meta-analysis

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Background. Laser protocols for the treatment of dentin hypersensitivity (DH) have not yet been studied systematically.

Objectives. The present study aimed to review clinical trials on the treatment of DH with laser therapy through a systematic review and meta-analysis.

Material and methods. The search of electronic databases resulted in 562 publications up to April 2020. The inclusion criteria were studies carried out on humans and reporting on the treatment of DH with laser therapy. Case reports, literature reviews and systematic reviews were excluded. Selected by abstract, potentially eligible papers were read in full ($n = 160$). Independent examiners performed data extraction and the assessment of the risk of bias.

Results. A total of 34 studies were included in the analysis, and 11 in the quantitative analysis. It was observed that most studies followed up patients for a maximum of 6 months (55%). Through the meta-analysis, we observed statistically significant differences between the average pain before and after 3 months of treatment with high- and low-power lasers. However, through indirect comparisons, it was observed that the high-power laser showed a greater tendency to reduce the pain levels after 3 months of treatment as compared to the low-power laser, but without a statistically significant difference.

Conclusions. It was possible to conclude that regardless of the type of laser used in the treatment of DH, this treatment is an effective option for the control of pain symptoms. However, it was not possible to establish a defined treatment protocol, since the evaluation methods are very different from each other.

Text for Rewiew and clinical cases.

Keywords: systematic review, laser, hypersensitivity, therapy, dentin

Introduction

Dentin hypersensitivity (DH) is one of the most commonly encountered clinical problems in dental practice. It is defined as pain resulting from exposed dentin in reply to chemical, thermal, tactile, or osmotic stimuli, which cannot be attributed to any other defect or dental pathology.[1–4] Clinically, it is a localized or generalized sharp pain of quick beginning and can affect one or several dental surfaces.[5] DH can have an adverse effect on the quality of life, especially concerning limitations of diet, tooth hygiene, and daily activities.[6–8] A significant association was found between DH and the frequency of tooth brushing in a recent study.[9] In addition, poor oral hygiene can result in the emergence of other oral diseases and amplification of DH.[9] Hypersensitivity in younger patients is often caused by the exposure of dentin due to erosion, while gingival recession often causes hypersensitivity in older patients due to the exposure of the dentinal tubules in the cervical areas caused by periodontal disease and intensified brushing activity.[10]

Many theories have been proposed to explain the biological mechanism of DH. For example, dentinal receptor mechanisms, odontoblastic transducer mechanisms, pain receptors on nerves in the pulp, nerves stimulated by hydrodynamic mechanisms, and nerve impulses modulated by the release of polypeptides.[11] However, the hydrodynamic system theory is the most accepted, and it suggests that the painful sensation is induced by the stimulation of dentin exposed by the movement of fluid within open dentin tubules in reply to mechanical or osmotic alterations/stimuli. The dislocation of fluid in the interior of the tubules causes intrapulpar pressure, which stimulates the nerve fibers located in the dentin-pulp interface and generates tooth pain.[12]

There is a range of treatment options for DH, such as toothpaste, potassium-based agents, glutaraldehyde-based agents, varnishes, strontium or acetate chlorides, resins, oxalates, calcium phosphates, and laser therapy.[13] All procedures work to obliterate the tubules and/or desensitize pulp receptors,[14] but none have produced predictable long-term results. This is thought to be because the physical and chemical processes in the oral environment can result in the tubules being exposed again. The ability to resist the recurrence of pain would be essential for laser therapy to maintain its long-term effectiveness.[15]

However, many studies have shown laser therapy to be an effective treatment for hypersensitivity.[16–19] The treatment can be accomplished with high or low-intensity laser therapies.[16,18–21] According to the wavelength of the laser, the action on the dentin surface during the treatment of DH is different depending on the irradiation parameters (mainly wavelength and energy density) and also on the time of treatment.[22]

The first mechanism of action of the high-power laser occurs by the obliteration of tubules through the partial fusion of dentin substrates.[6,23,24] The low-power laser decreases the chronic inflammatory process present in the cells, by activating the sodium and potassium pumps and obliterating dentin tubules through the formation of tertiary dentin,[25] and can promote analgesic, anti-inflammatory, and biomodulatory effects.[17,22] Laser treatment is well tolerated by patients and is considered safe, quick, and painless.[26]

A systematic review in 2013 by Lin et al. showed that the different desensitizing treatments used in the office had significantly better results than placebo.[27] However, these authors did not stratify the results according to the type of laser evaluated. In the same year, another systematic review showed that both lasers – high and low-power – showed effective results in the treatment of DH.[16]

In contrast, until now, there are no established treatment protocols in the literature for DH with laser therapy and its long-term effectiveness. Considering these facts, this study aimed to review clinical trials and protocols for DH treatment with laser therapy through a systematic review and meta-analysis, in addition to updating the studies available on the proposed subject.

Methods

This article was written using the PRISMA protocol as a guide for the systematic review available at http://www.prisma-statement.org and was registered in the PROSPERO database (CRD42018095822).

Search strategy and selection criteria

The search was performed and limited to studies in PubMed (MEDLINE) with an English language restriction up to April 2020. The literature search was conducted as displayed in Fig. 1.

The selection was conducted by two authors (LAP and LLMM). Each author reviewed the title and abstract of the papers and selected the studies based on inclusion criteria. Disagreements on studies were resolved by a discussion, with the involvement of a third review author when necessary. They were trained to achieve nearly perfect agreement of the Kappa index.

Studies were selected according to the criteria defined below.

((((((((Lasers[MeSH Terms]) OR laser*[Text Word]) OR Laser therapy[MeSH Terms]) OR "Photodynamic therapy"[Text Word]) OR "Daylight pdt"[Text Word])) AND ((((((((((clinical trial[MeSH Terms]) OR "clinical trial"[Text Word]) OR trial*[Text Word]) OR Therap*[Text Word]) OR Therapeutics[MeSH Terms]) OR Therapy[MeSH Terms]) OR "Operative dentistry"[Text Word]) OR Decision making[MeSH Terms]) OR Treat*[Text Word]) OR manage*[Text Word]))) AND ((((((Dentin[MeSH Terms]) OR Dentin*[Text Word]) OR Teeth[Text Word]) OR tooth[Text Word]) OR Dentition[MeSH Terms]) OR Dentition*[Text Word]) OR dental[Text Word])) AND ((((Hypersensitivity[MeSH Terms]) OR Hypersensit*[Text Word]) OR Dentin sensitivity[MeSH Terms]) OR Sensitivit*[Text Word])

Fig. 1. Search strategy

The inclusion criteria were: (1) studies carried out on humans; (2) articles that reported on the treatment of DH using laser therapy; and (3) studies that were not case reports, literature reviews, or systematic reviews.

The full text of all eligible articles was subject to the following exclusion criteria: (1) non-randomized clinical trials; (2) not written in the English language; (3) not in permanent teeth; (4) studies without laser therapy treatment; (5) studies that did not have at least 3 months of follow-up after baseline; and (6) studies without sufficient information for data extraction.

Data extraction

The studies that met the inclusion and exclusion criteria were submitted for data extraction. The information was extracted by an author (LAP) and later verified by a second author (LLMM). Both reviewed articles at all stages, and differences were discussed until a consensus was reached.

A standardized data extraction form was used to record the following details:

– study characteristics (author, publication year, title, publication journal, study design, country, setting, funding);
– number of teeth treated;
– participant age;
– study design;
– endpoint assessment of pain;
– follow-up time;
– drop-outs in follow-up;
– mean and standard deviation (*M* ±*SD*) values before and after laser therapy treatment – second outcome measures;
– success of intervention judged by pain reduction;
– information about the treatment protocol, such as laser type (high or low-power), application time, number of sessions applied, application range, and laser power and energy – primary outcome measures; and
– adverse events.

If there was not enough information described in the paper, an email was sent to the authors in an attempt to complete the data extraction before it was excluded from the review.

The primary endpoint of interest was to research the protocols existing for DH treatment using laser therapy. The secondary endpoint was to try to verify which DH treatment using laser therapy was more successful through the meta-analysis.

Assessment of the risk of bias

Each selected study was assessed qualitatively using the Cochrane Handbook for the Development of Systematic Intervention Reviews, v. 5.1.0 (Cochrane Handbook). The criteria were divided into main domains associated with randomization, blinding, outcome data, and sample characteristics at baseline. The bias risk assessment was accessed as 'low risk of bias, 'high risk of bias, or 'confuse/unclear' when it was not possible to identify the information or it was uncertain about the subject or potential bias (classified based on each item of the study criteria). Both reviewers (LAP and JJF) performed independent analyses and disagreement was resolved via a discussion.

Statistical analysis

Direct comparisons were performed between the two treatments using a meta-analysis of random effects. Also, network meta-analyses were conducted to allow indirect comparison between high and low-power lasers. We conducted a Bayesian analysis of mixed treatment comparisons (MTC) to concurrently consider both direct and indirect signs. Studies were performed in similar groups of patients, meeting the assumption of transitivity of this network analysis. First, MTC analyses were computed using both fixed and random models, and the merit of fit for the models was quantified using the residual deviation and the residue information criterion (RIC), with lower scores being better. Because of that, the random effects model with consistent variability among studies was chosen for the final MTC analysis. A node split analysis for unreliability was performed for the pairs with both direct and indirect evidence.

Meta-analyses and network meta-analyses were performed using the R package (GeMTC), v. 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). For each involved study, mean differences (*MD*s) and 95% confidence intervals (*CI*s) were estimated.

Results

Study selection

The inter-examiner reproducibility (Kappa index) was 0.85 and considered excellent. The systematic search of the literature identified 562 potentially relevant studies. After removing duplicate studies and screening the titles, abstracts, and/or full texts, 34 studies were included and analyzed qualitatively.

The main reason for not including these studies in the review was that they were not performed on humans (in vivo study, 40%). The remaining 160 studies were analyzed using the full texts for further details and study judgment. Finally, 34 articles matched the eligibility criteria and had adequate data for qualitative analysis, and 11 articles were included in the meta-analysis. The details of the bibliographic research and the selection process of the studies are illustrated in Fig. 2.

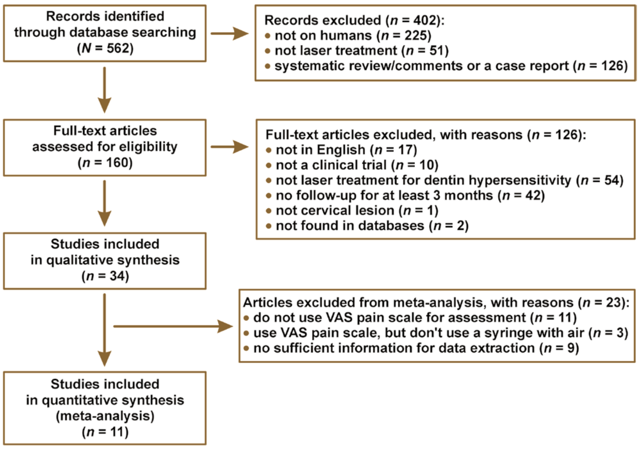

Fig. 2. PRISMA flowchart of the literature search, determination of eligibility, and inclusion in the systematic review and meta-analysis

Study design

The main characteristics of our data sets of longitudinal studies are shown in Table 1.

It was observed that most studies had a split-mouth design (55%), and the age of these subjects ranged from 11 to74 years.

Most of the criteria used for DH assessment and discomfort were reported by the patient. The use of air from a triple syringe followed by the application of a visual probe to scratch the dental surface or perform a thermal test. However, it was also observed that there was no standardization regarding the pain scale used, but most studies use the visual analog scale (VAS) – 70%. The VAS was a 10 cm extension and, with each side, contained an indication of 'no pain' and 'severe pain.'

Table 1. Characteristics of the clinical trials on the laser therapy for dental hypersensitivity (DH) selected for qualitative analysis (= 34)

Author (year)	Number of teeth	Participant age [years]	Study design	Treatment protocol	End point Assessment of pain	Follow-up time [months]	Drop-outs (%)
Moura et al. (2019)[28]	60	18	Parallel groups	-GaAlAS laser	Air stimulus + VAS scale	6	0
El Mobadder et al. (2019)[29]	Not reported	Not reported	Single arm	- Diode laser	Air stimulus +water + VAS scale	6	3.2
Yadav et al. (2019)[30]	Not reported	Not reported	Parallel groups	- Nanocrystalline hydroxypatite + Nd:YAG + scaling and root planning (SRP) - SRP + Nd:YAG + herbal dentifrice - SRP + Nd:YAG	Air stimulus +water + VRS scale	6	Not reported
Narayanan et al. (2019)[31]	Not reported	18-60	Parallel groups	- Diode laser - Diode laser + potassium nitrate desensitising paste	Air + thermal + tactile stimulus + VAS scale	3	0
Tabatabaei et al. (2018)[32]	135	25-58	Parallel groups	- Diode laser - Nd:YAG	Thermal and not reported scale	6	0
BouChebel et al. (2018)[33]	54	20-60	Split-mouth	- Nd:YAG	Air, tactile, thermal stimulus + VAS scale	6	0
Praveen et al. (2018)[34]	50	20-50	Parallel groups	- Diode laser	Air + water stimulus + VAS scale	3	0
Douglas-de-Oliveira et al. (2018)[35]	434	12-60	Split-mouth	- GaAlAS laser	Air stimulus + VAS scale	6	0
Ozlem et al. (2018)[36]	100	18-56	Parallel groups	- Nd:YAG - Gluma desensitizer (DG) + Nd:YAG - Er,Cr:YSGG - DG + Er,Cr:YSGG	Probe (Tactilstimulus) + Pain yes or no	6	0%
Lopes et al. (2017)[17]	117	22-53	Parallel groups	- Low-power laser, low parameters (LPLD) - Low-power laser, high parameters (LPHD) - LPLD + DG - LPHD + DG - Nd:YAG laser - Nd:YAG laser + DG - LPLD + Nd:YAG - LPHD + Nd:YAG	Air stimulus + probe + VAS scale	18	Not reported
Lima et al. (2017)[37]	434	12-60	Slipt-mouth	- Diode laser	Air stimulus + VAS scale	3	0%
Lopes et al. (2015)[38]	55	22-53	Parallel groups	- LPLD - LPHD - LPLD + DG - LPHD + DG	Air stimulus + VAS scale	6	0%

Author (year)	Number of teeth	Participant age [years]	Study design	Treatment protocol	End point Assessment of pain	Follow-up time [months]	Drop-outs (%)
Bal et al. (2015)[39]	156	19-60	Slipt-mouth	- Diode laser - Diode laser + Sensitivity paste - Sensitivity paste + Diode laser	Air stimulus + VAS scale	6	0%
Talesara et al. (2014)[40]	80	25-55	Slipt-mouth	- Nd:YAG laser	Air stimulus + thermal test + VAS scale	9	0%
Femiano et al. (2013)[41]	262	21-64	Slipt-mouth	- Diode laser - NaF + Diode laser	Air stimulus + VAS scale	6	0%
Lund et al. (2013)[42]	117	19-58	Slipt-mouth	- Diode laser	Air stimulus + VAS scale	3	0%
Flecha et al. (2013)[43]	432	Not reported	Slipt-mouth	-GaAIAs laser	Air stimulus + number scale	6	0%
Lopes et al. (2013)[44]	33	Not reported	Split-mouth	-Nd:YAG laser -Nd:YAG laser + DG	Air stimulus + probe + VAS scale	6	0%
Ehlers et al. (2012)[45]	181	Not reported	Split-mouth	- Er,Cr:YSGG laser	Air stimulus + VAS scale	6	21%
Yilmaz et al. (2011)[25]	174	18-60	Split-mouth	-Diode laser - Er,Cr:YSGG laser	Air stimulus + VAS scale	3	0%
Yilmaz et al. (2011)[46]	48	11-58	Split-mouth	-GaAIAs laser	Air stimulus + VAS scale	6	0%
Yilmaz et al. (2011)[47]	146	Not reported	Split-mouth	- Er, Cr: YSGG	Air stimulus + VAS scale	3	0%
Vieira et al. (2009)[48]	164	24-68	Parallel groups	- Diode laser	Air stimulus + probe + VAS scale	3	20%
Ipci et al. (2009)[49]	420	23-62	Parallel groups	- CO2 laser - ER: YAG laser - CO2 laser + NaF - ER:YAG laser+ NaF	Air stimulus + Ushida method	6	0%
Aranha et al. (2009)[50]	101	Not reported	Parallel groups	- Diode laser	Air stimulus + VAS scale	6	Not reported
Birang et al. (2007)[51]	63	Not reported	Split-mouth	- Nd:YAG laser - Er:YAG laser	Probe + VAS scale	6	0%
Ciaramicoli et al. (2003)[52]	145	23-63	Parallel groups	-Nd:YAG laser	Air stimulus + probe + pain scale	6	Not reported
Lier et al. (2002)[53]	34	26-66	Split-mouth	-Nd:YAG laser	Air stimulus + VAS scale	4	0%
Schwarz et al. (2002)[54]	208	23-56	Split-mouth	- Er:YAG laser	Air stimulus + Pain scale (4 degrees)	6	0%
Zhang et al. (1998)[55]	91	25-74	Single arm	-CO2 laser	Air stimulus + pain scale (3 degrees)	3	0%
Moritz et al. (1998)[56]	144	Mean 37-45	Parallel groups	-CO2 laser + strontium chloride	Air stimulus + probe + Pain scale (4 degrees)	18	Not reported
Gutknecht et al. (1997)[57]	120	26-62	Split-mouth	-Nd:YAG laser	Air stimulus + probe + Pain scale (3 degrees)	3	Not reported
Lan et al. (1996)[58]	34	26-66	Split-mouth	-Nd:YAG laser	Air stimulus + VAS scale	4	11%
Gelskey et al. (1993)[59]	19	25-69	Split-mouth	-Helium neon laser -Helium neon laser + Nd:YAG laser	Mechanical and thermal stimulus + VAS scale	3	5%

GaAlAs – gallium-aluminum-arsenide; Nd:YAG – neodymium-doped yttrium aluminum garnet; SRP – scaling and root planing; DG – Gluma desensitizer; Er,Cr:YSGG – erbium, chromium-doped yttrium, scandium, gallium garnet; LPLD – low-power laser, low parameters; LPHD – low-power laser, high parameters; NaF – fluoride sodium; CO2 – carbon dioxide; VAS – visual analog scale.

Follow-up lengths were most frequently 6 months (55%), and only two studies followed the patients for a period of 18 months.

The qualitative analysis included 63 treatment groups, and Table 2 shows the main characteristics of the laser therapy protocols amongst each group, which are described below.

Table 2. Main features of the laser therapy protocols for each group (*n* = 51)

Author (year)	Laser type	Potency	Potency [W/mW]	Energy density [J/cm^2]	Wavelength [nm]	Application time [s]	Same-day sessions	Application interval time on different days	Number of repetitions on different days	Comments
Yadav et al. (2019)[30]	Nanocrystalline hydroxyapatite + Nd:YAG + SRP	High	1W	Not reported	Not reported	60	5to 20	1 week, 1 month, 6 months	4	Nano crystalline hydroxyapatite 2x/day
Yadav et al. (2019)[30]	SRP + Nd:YAG + herbal dentifrice	High	1W	Not reported	Not reported	60	5to 20	1 week, 1 month, 6 months	4	Herbal dentifrice 2x/day
Yadav et al. (2019)[30]	SRP + Nd:YAG	High	1W	Not reported	Not reported	60	5to 20	1 week, 1 month, 6 months	4	-
Tabatabaei et al. (2018)[32]	Nd:YAG	High	1W	Not reported	1064	30	1	1 week	3	-
BouChebel et al. (2018)[33]	Nd:YAG	High	0.64W	100	Not reported	20	4	-	1	-
Ozlem et al. (2018)[36]	Nd:YAG	High	1W	35.8	Not reported	20	3	-	1	-
Ozlem et al. (2018)[36]	DG + Nd:YAG	High	1W	35.8	Not reported	20	3	-	1	30-60sec DG application after laser therapy
Ozlem et al. (2018)[36]	Er,Cr:YSGG	High	0.25W	44.3	Not reported	30	1	-	1	
Ozlem et al. (2018)[36]	DG + Er,Cr:YSGG	High	0.25W	44,3	Not reported	30	1	-	1	30-60sec DG application after laser therapy
Lopes et al. (2017)[17]	Nd:YAG	High	1W	85	Not reported	15	4	-	1	-
Lopes et al. (2017)[17]	Nd:YAG + DG	High	1W	85	Not reported	15	4	-	1	30-60sec DG application after 3rd laser therapy
Talesara et al. (2014)[40]	Nd:YAG	High	1W	Not reported	Not reported	60	2	-	1	-
Lopes et al. (2013)[44]	Nd:YAG	High	1.5W	85	Not reported	60	4	-	1	-
Lopes et al. (2013)[44]	Nd:YAG + DG	High	1.5W	85	Not reported	60	4	-	1	30-60sec DG application after 3rd laser therapy
Ehlers et al. (2012)[45]	Er,Cr:YSGG	High	0.001W	0.06	665	Not reported	1	-	1	-
Yilmaz et al. (2011)[25]	Er,Cr:YSGG	High	0.25W	Not reported	2780	30	1	-	1	-
Yilmaz et al. (2011)[47]	Er,Cr:YSGG	High	0.25W	Not reported	Not reported	30	1	-	1	-
Ipci et al. (2009)[49]	CO2 laser	High	1W	Not reported	Not reported	10	1	-	1	-
Ipci et al. (2009)[49]	Er:YAG laser	High	Not reported	60	Not reported	10	1	-	1	-
Ipci et al. (2009)[49]	CO2 laser + NaF	High	1W	Not reported	Not reported	10	1	-	1	NaF applied for 4 min

Author (year)	Laser type	Potency	Potency [W/mW]	Energy density [J/cm²]	Wavelength [nm]	Application time [s]	Same-day sessions	Application interval time on different days	Number of repetitions on different days	Comments
Ipciet al. (2009)[49]	Er:YAG laser + NaF	High	Not reported	60	Not reported	10	1	-	1	NaF applied for 4 min
Birang et al. (2007)[51]	Er:YAG laser	High	Not reported	100	Not reported	60	2	-	1	-
Birang et al. (2007)[51]	Nd:YAG laser	High	1W	Not reported	Not reported	60	2	-	1	-
Ciaramicoli et al. (2003)[52]	Nd:YAG	High	1W	0.04	1064	30	1	1 week	3	-
Lier et al. (2002)[53]	Nd:YAG laser	High	4W	Not reported	Not reported	120	1	-	1	-
Schwarz et al. (2002)[54]	Er:YAG laser	High	Not reported	80	Not reported	120	1	-	1	-
Zhag et al. (1998)[55]	CO2 laser	High	1W	Not reported	Not reported	0,5	3	-	1	-
Moritz et al. (1998)[56]	CO2 laser + strontium chloride	High	0.5W	Not reported	Not reported	5	6	-	1	The number of laser applications varies according to the pain reported by the patient
Gutknecht et al. (1997)[57]	Nd:YAG laser	High	0.3W	Not reported	1064	30-90	1	-	1	-
Gutknecht et al. (1997)[57]	Nd:YAG laser	High	0.6W	Not reported	1064	30-90	1	-	1	-
Gutknecht et al. (1997)[57]	Nd:YAG laser	High	1W	Not reported	1064	30-90	1	-	1	-
Lan et al. (1996)[58]	Nd:YAG laser	High	Not reported	30	Not reported	120	1	-	1	-
Moura et al. (2019)[28]	GAIAs laser	Low	100mW	4	808	40	4	48h	4	-
El Mobadder et al. (2019)[29]	Diode laser	Low	1000mW	Not reported	980	Variable	Not reported	-	1	-
Narayanan et al. (2019)[31]	Diode laser	Low	1000mW	Not reported	810	10	Not reported	Not reported	Not reported	-
Narayanan et al. (2019)[31]	Diode laser + potassium nitrate	Low	1000mW	Not reported	810	10	Not reported	Not reported	Not reported	-
Tabatabaei et al. (2018)[32]	Diode laser	Low	200mW	84.4	810	30	1	1 week	3	-
Praveen et al. (2018)[34]	Diode laser	Low	60mW	9	905	60	1	-	1	-
Douglas-de-Oliveira et al. (2018)[35]	GaAIAS laser	Low	120mW	30.9	795	8	1	48h	3	-
Lopes et al. (2017)[17]	LPLD	Low	30mW	10	Not reported	9	1	72h	3	-
Lopes et al. (2017)[17]	LPHD	Low	100mW	40	Not reported	11	1	72h	3	-
Lopes et al. (2017)[17]	LPLD + DG	Low	30mW	10	Not reported	9	1	72h	3	30-60sec DG application after 3rd laser therapy
Lopes et al. (2017)[17]	LPHD + DG	Low	100mW	40	Not reported	11	1	72h	3	30-60sec DG application after 3rd laser therapy
Lima et al. (2017)[37]	Diodo laser	Low	120mW	30.96	795	8	1	48h	3	-

Author (year)	Laser type	Potency	Potency [W/mW]	Energy density [J/cm²]	Wavelength [nm]	Application time [s]	Same-day sessions	Application interval time on different days	Number of repetitions on different days	Comments
Lopes et al. (2015)[38]	LPLD	Low	30mW	10	635-685	9	1	72h	3	-
Lopes et al. (2015)[38]	LPHD	Low	100mW	90	808-830	11	1	72h	3	-
Lopes et al. (2015)[38]	LPLD + DG	Low	30mW	10	635-685	9	1	72h	3	30-60sec DG application after 3rd laser therapy
Lopes et al. (2015)[38]	LPHD + DG	Low	100mW	90	808-830	11	1	72h	3	30-60sec DG application after 3rd laser therapy
Bal et al. (2015)[39]	Diodo laser	Low	25mW	2	685	100	1	-	1	-
Bal et al. (2015)[39]	Diodo laser+ toothpaste for sensitivity	Low	25mW	2	685	100	1	-	1	Applying the toothpaste after laser therapy 2 times one after the other
Balet al. (2015)[39]	Toothpaste for sensitivity+ Diodo laser	Low	25mW	2	685	100	1	-	1	Applying the toothpaste after laser therapy 2 times one after the other
Femiano et al. (2013)[41]	Diode laser	Low	200mW	-	808	60	1	168h	3	-
Femiano et al. (2013)[41]	NaF + Diode laser	Low	200mW	-	808	60	1	168h	3	NaF applied for 60 sec
Lund et al. (2013)[42]	Diode laser	Low	20mW	5	780	40	4	72h	3	-
Flecha et al. (2013)[43]	GAlAs laser	Low	120mW	2.88	785	8	1	48h	3	-
Yilmaz et al. (2011)[46]	GAlAs laser	Low	500mW	5	810	60	1	-	1	-
Yilmaz et al. (2011)[25]	Diodo laser	Low	Not reported	8.5	810	60	1	-	1	-
Vieira et al. (2009)[48]	Diodo laser	Low	30mW	4	660	120	1	168h	4	-
Aranha et al. (2009)[50]	Diodo laser	Low	15mW	3.8	660	10	1	72h	3	-
Gelskey et al. (1993)[59]	Helium neon laser	Low	Not reported	30	632	10	Varies	-	1	The number of laser applications varies according to the pain reported by the patient
Lopes et al. (2017)[17]	LPLD + Nd:YAG	Low/high	30mW/1W	10/85	635-685/400000	9/15	¼	72h/0	3/1	Nd:YAG laser application on 3rd session
Lopes et al. (2017)[17]	LPHD + Nd:YAG	Low/high	100mW/1W	40/85	808-830/400000	11/15	¼	72h/0	3/1	Nd:YAG laser application on 3rd session
Gelskey et al. (1993)[59]	Helium neon laser + Nd:YAG laser	Low/high	Not reported	30	632/1064	10	Varies	-	1	The number of laser applications varies according to the pain reported by the patient

Most of the protocols using lasers with or without another desensitizing agent reduced the pain levels of the patients during the follow-up period. Moreover, the studies that evaluated patients for 18 months had significant results regarding a decrease in pain for all treatments.

The low-power diode laser was the device of choice in 49% of groups that used the low-intensity laser alone or in combination with other therapies. The high-power laser was used alone or combined with other therapies in 55% of the groups that used high-intensity lasers.

It was observed that the average application time for the groups that used a high-power laser was 43 s and that it varied from 1 to 20 applications on the same day. On the other hand, groups using this type of laser did not repeat the treatment protocol on different days, except two studies performed the protocol for three days at intervals of one week per application, and one study performed the protocol on four different days (baseline, 1 week, 1 month, and 6 months). In addition, 31% of the groups evaluated used high-power laser therapy with a desensitizing agent.

In the low-power laser groups, the average application time was 36 s, not exceeding 1 application per day, but being able to repeat applications on different days (maximum 4 applications), except in two groups that performed 4 applications, each lasting 10 s on the same day. Only 9 groups evaluated the use of low-power laser therapy with a desensitizing agent or desensitizing toothpaste.

Risk of bias

The details of the bias risk assessments for each study are presented in Fig. 3. Most studies had one or more "confused/unclear" classifications due to a lack of information. In 40% of the studies, the random sequence generation was described in detail, however, of these, only 4 described the allocation concealment method.

In 68% and 65% of the studies with a low risk of bias had blinding of participants/personnel and outcome assessments, respectively. Regarding incomplete outcome data, the majority had no attrition levels above 20% at the end of the follow-up.

Also, 34% had a high risk of bias concerning the other bias items, as they varied in the number of laser applications administered to the same patient or indicated the use of fluoride-free toothpaste during treatment. In addition, some had a flaw in the methodology that the authors deemed important.

Regarding the articles that were analyzed using the meta-analysis, only 1 did not describe the randomization sequence, 2 did not describe the allocation concealment, and 2 were at high risk due to other biases.

Two studies are not represented in the graph, as they presented only one treatment arm, making it impossible to assess the risk of bias.

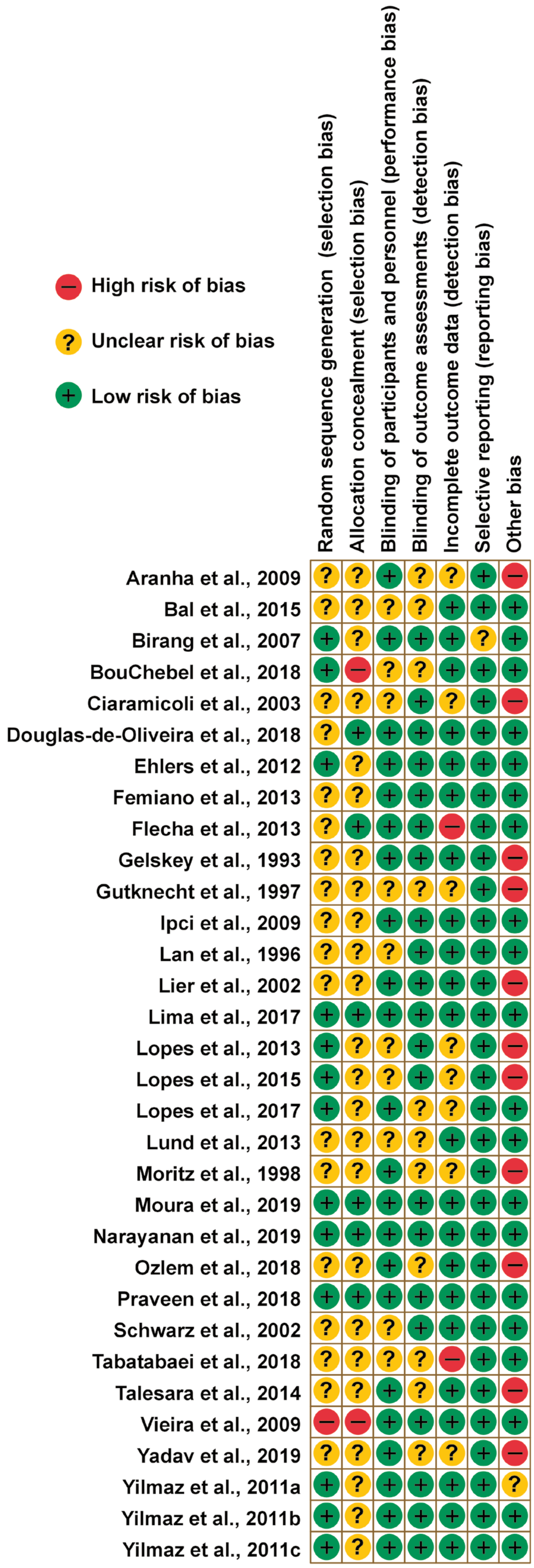

Fig. 3. Methodological quality and the risk of bias of longitudinal clinical trials selected for the study

Red, green and yellow colors refer to a high risk of bias, a low risk of bias and an unclear risk of bias, respectively.

Meta-analysis

Only the articles that used the VAS with a syringe of air to assess DH were used for the meta-analysis to standardize the patient's pain collection. The groups were separated between high and low-intensity lasers using the power described in watts (W) or milliwatts (mW) to perform the meta-analysis separately.

Nine articles were not included in the analysis due to not providing sufficient information about the means and standard deviations before and after treatments. Groups that used desensitizing agents in conjunction with laser therapy were also not included in the meta-analysis.

Direct comparisons between the groups

There was a significant difference between average pain levels before and after 3 months of treatment when compared to a treatment control group (before the treatment), regardless of the type of laser used (Fig. 4).

Indirect comparisons between the groups

Through indirect comparisons, it was observed that the high-power laser showed a greater tendency to reduce pain levels after 3 months of treatment when compared to the low-power laser, but without a statistically significant difference (Table 3 and Fig. 5). Indirect comparisons did not influence the results for low-power versus no treatment and high-power versus no treatment. In that way, there was no inconsistency.

Ranking and network analyses

In Fig. 6, it was possible to observe through the ranking and network analysis that the high-power lasers showed a greater chance to be the better treatment option, followed by low-power lasers, and performing no treatment the worst concerning self-reported pain for DH. The network diagram is also shown in Fig. 6.

Discussion

Various techniques and different protocols for the treatment of DH have been described in the literature, but no systematic reviews and meta-analyses have been performed to date to evaluate DH treatment protocols. A full search strategy was used, though, to decrease the risk of bias, only randomized controlled trials were chosen for this systematic review.

After including the eligible studies, a qualitative analysis of 34 studies was carried out. In contrast, due to the lack of standardization in pain assessments and the lack of information necessary to perform a quantitative analysis, only 11 studies were considered for the meta-analysis.

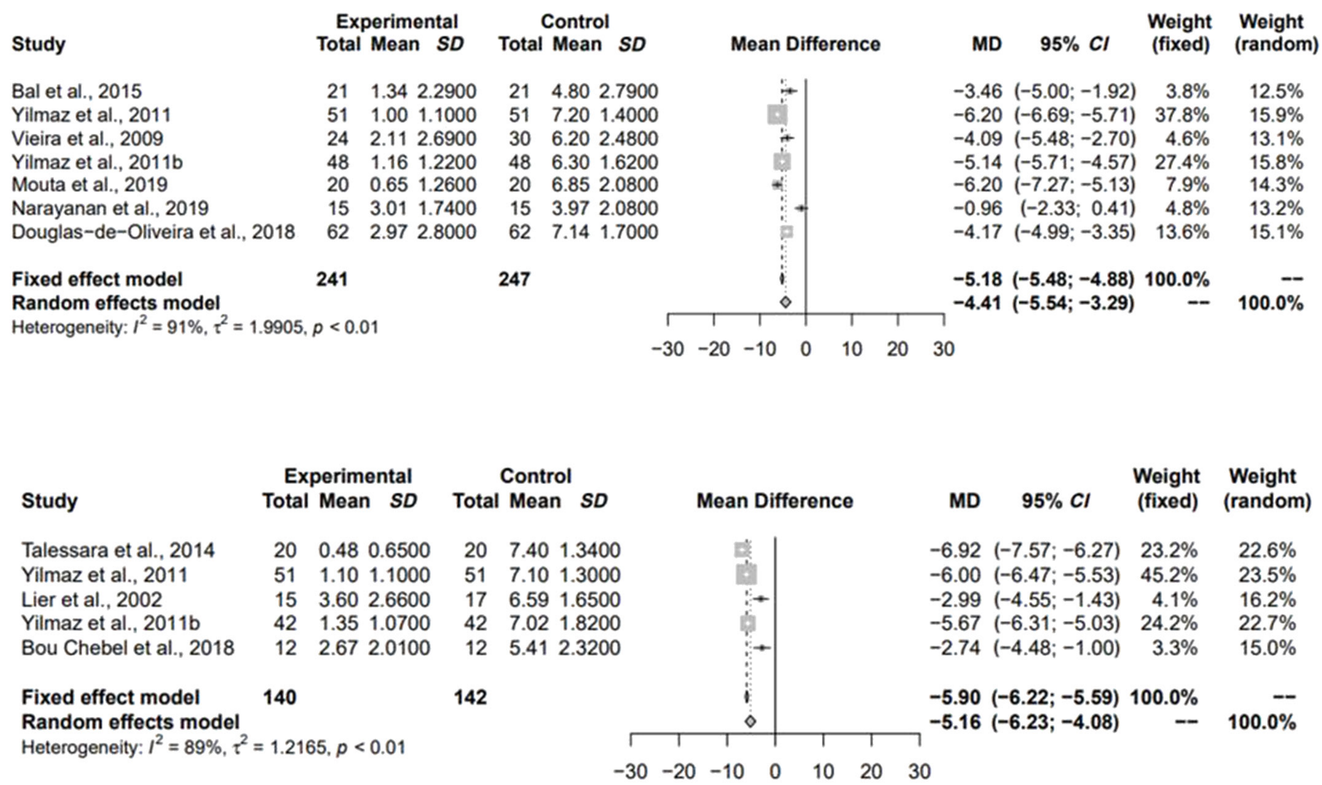

Fig. 4. Forest plot of comparison: Pain reported by the patient before and after 3 months of treatment with a low-power laser and a high-power laser respectively

Table 3. Indirect comparisons between the groups

	Direct evidence *MD* (95% *CI*)	MTC (direct + indirect evidence) *MD* (95% *CI*)
High vs. none	5.0 (3.0–6.8)	5.0 (3.0–6.8)
Low vs. none	4.4 (2.8–6.0)	4.4 (2.8–6.0)
High vs. low	–	0.62 (−1.9–3.1)

MD – mean difference; *CI* – confidence interval; MTC – mixed treatment comparison: high laser, none – before the treatment, low laser.

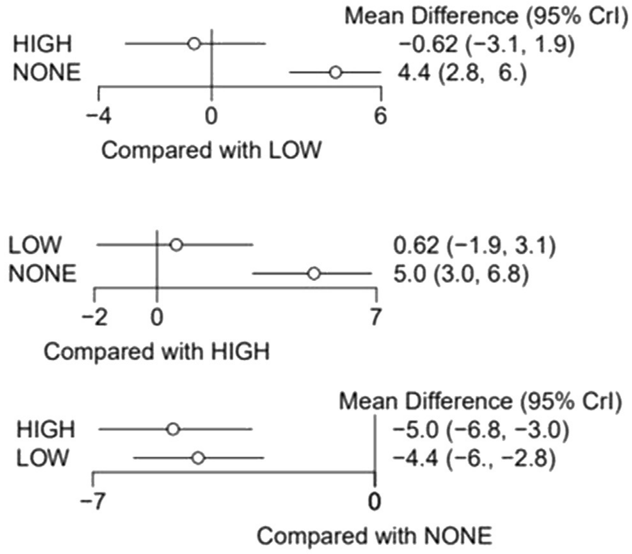

Fig. 5. Forest plot of indirect comparisons between the groups

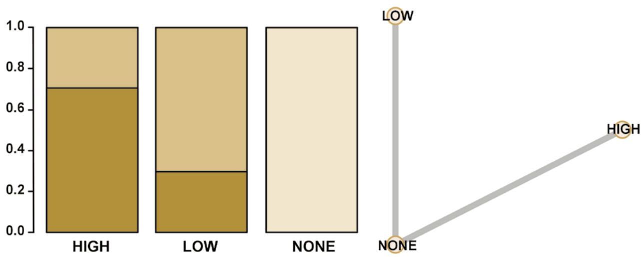

Fig. 6. Ranking and network analyses

Through the meta-analysis, it was possible to verify that the average pain reported by patients was lower after 3 months of follow-up when compared to the average pain before treatment with laser therapy.

The management and treatment of DH are complicated as a consequence of their multifactorial nature, and factors such as parafunctional habits, diet, hygiene, and periodontal and pulp health can influence the results, making research about DH complex and challenging.[60] Recently, an improvement in laser technology has allowed laser therapy to emerge as a new treatment alternative for DH.[61] There are several methods to assess DH intensity, but the lack of standardization in pain measurements is a difficulty faced by researchers.[62,63] This can be explained by the fact that the perception of each individual's pain is very subjective, which depends directly on the individual's ability to define what he is feeling through numerical scales.[64] The index most used in the studies and evaluated in the qualitative analysis for pain was the VAS (70%).

Various stimuli can be used, such as tactile stimuli using probes and thermal stimuli using a cold water jet and air spray.[65] Differences in the mode of DH assessment can also lead to discrepancies in studies, contributing to the heterogeneity of painful responses. Compared to tactile stimulation, air spray is the most reliable method for assessing DH.[66] The meta-analysis of this study included only treatment groups that assessed pain by air-jet using a triple syringe, followed by the application of the VAS in an attempt to standardize some parameters to the maximum.

Other variables, such as the wavelength, power, and energy generated by the laser device, are considered of extreme importance since their variability can directly interfere with their applicability. However, it was also observed that these parameters varied among studies, and most high-power laser studies have not described the wavelength and energy used. Which is different from the groups treated with low-power lasers, which described these main parameters. This can be explained since most high-power lasers have pre-established wavelengths depending on the model of the device, and this value can only vary in low-power models.

Split-mouth studies are preferred in dentistry, as it is possible to minimize inter-individual variables, being the participants are their controls, and when these variables are present, they can interfere in the same way with the treatments. However, in cases where it is necessary to assess the patient's pain on both sides of the mouth, one of the responses may be influenced by the pain the patient is experiencing on the other side of the mouth. It is important to note that most works in this systematic review were carried out using this form.

The low-power lasers used for the treatment of DH can interact with the dental pulp leading to an increase in odontoblastic activity and, consequently, the production of tertiary dentin and a decrease in the lumen size of dentinal tubules. This type of laser can also stimulate cell activity, promote anti-inflammatory effects, and produce analgesic effects.[5,17,67] High-power lasers, such as carbon dioxide (CO_2) and neodymium-doped yttrium aluminum garnet (Nd:YAG), are the typical types of lasers used in dentistry. These high-power lasers can obliterate the dentinal tubules through fusion and re-solidification by melting the hydroxyapatite and, after cooling, solidifying and forming hydroxyapatite crystals larger than in the initial structure, thus reducing DH.[44,68]

Most of the studies evaluated, regardless of the type of laser used, showed satisfactory results regarding DH pain after months of therapy. The low-power laser most used by the authors was the diode laser. It is known that, in addition to having an immediate analgesic effect, it stimulates odontoblasts in the production of irregular dentin that can lead to the obliteration of dentinal tubules,[69,70] and provide analgesia after months of treatment.

Some authors have reported the disadvantages of lasers, including that they are very expensive, intricate to use, and use over time decreases, among others.[5] However, new research shows that laser therapy is well tolerated by patients and is safe and effective.[26,38] After performing the meta-analysis of treatments with high and low-power lasers, it was observed that both lasers showed a reduction in DH after 3 months of treatment by alleviating symptoms through the formation of reactionary dentin after irradiation.[52,71]

In this work, through indirect comparisons, it was found that the high-power laser had lower average pain scores after 3 months of treatment when compared to the low-power laser or with the average pain before treatments, however, there was no difference between them. But, management for DH with laser therapy in the office constitutes a more expensive and elaborate treatment modality than traditional treatment planning,[5] and high-power equipment has a higher cost when compared to low-power equipment. Therefore, as the results of high and low-power lasers are similar, the low lasers seem to be a viable option concerning the cost for the clinician, such as the diode laser. Besides that, no study has evaluated their cost-effectiveness and more long-term studies are needed.

As a limitation of this study, it can be observed that the DH assessment methods are very different among studies, generating heterogeneous responses concerning pain, not only because the instrument of each study is different, but also because pain is a subjective and personal experience. In addition, some studies did not report the complete protocol used for each type of laser, often not being possible to include them in the analyses. Although most studies followed patients for 6 months after treatment (55%), it was decided to perform the meta-analysis with a 3-month follow-up to include more treatment groups in the quantitative analysis.

Regardless of the type of laser used in the treatment of DH, laser therapy is a proven choice for the control of pain symptoms, but it is not possible to establish a defined treatment protocol since the evaluations of each method are very different among the studies in this review. Also, the available signs suggest that high and low-power lasers are effective in decreasing DH after 3 months of treatment.

Conclusions

Laser therapy can be an effective choice for the control of pain symptoms, despite the different lasers and protocols studied for HD. According to the studies, it is suggested that lasers are effective in decreasing DH pain after 3 months of treatment.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets and all supplementary materials 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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Review

Reasons for the fatigue of ball attachments and their O-rings: A systematic review

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Abstract

The ball attachments and their O-rings used for the retention and stabilization of overdentures showed a decrease in retention as the number of cycles increased. This fact resulted in a decrease in the retention of the prosthesis. The purpose of this study was to evaluate the fatigue resistance of ball attachments through a systematic review. An electronic search was performed using the Cochrane Library, LILACS, PubMed, ScienceDirect, and Web of Science databases. The search was conducted based on the PICOS framework. The inclusion criteria involved in the search comprised research articles written in English and published between the years 2000 and 2020. In the final selection, 18 articles were included in the review. Most of these studies performed the fatigue retention tests using parallel implants without angles. However, some studies used different angles to analyze the fatigue retention values. With the passage of time, the wear results in deformation and, as a consequence, a decrease in the retention of most attachments, leading to treatment failure. The main factor to be considered is the loss of retention of these components and their low durability. The loss of retention is due to large extent to the materials used to manufacture the attachments and O-rings, the size and angulation of the implants, and the length of the prosthesis. Future research is needed to further elucidate the reasons for the failure of the attachments.

Keywords: fatigue, dental implants, overdenture, dental abutment

Introduction

Dental implants are used to rehabilitate patients who have suffered from the loss of dental elements.[1,2] However, due to the chronic and continuous resorption process of the residual alveolar ridge, the installation of dental implants is limited due to reduced bone thickness or height and the need to perform bone grafts, increasing the number of surgical steps and making the treatment more expensive and difficult for patients to access, either because of purchasing power or medical problems.[3,4] Also, factors such as bone density, age, smoking habit, and systemic osteometabolic diseases can negatively affect the primary stability of the implant and lead to early loss or even contraindicate its installation.[2,5,6]

Primary stability is important to perform the immediate installation of the prosthesis, reduce the number of surgical steps and trauma, as well as to improve the aesthetic and psychology of patients.[2,7–9] To achieve good primary stability and a good prognosis, different factors must be taken into account.[2,10,11] In 2021, Tardelli et al. evaluated the micro and microstructure of different implants and demonstrated that different geometries produce different results in primary stability and stress distribution.[11] While in 2020, Antonelli et al. evaluated the influence of different surgical protocols on the achievement of primary stability in cancellous bone.[2] Thus, taking these factors into account can improve the prognosis of the treatment using dental implants by reducing the probability of implant loss and, consequently, revision surgery with prostheses to support the dental implants.

Different modalities of rehabilitation for edentulous patients are described, among them, overdentures and fixed prostheses stand out.[12–16] The overdentures are removable dentures that can be supported over teeth or dental implants and have good long-term success rates in rehabilitating patients suffering from total edentulism, especially of the mandible,[17–20] where the problems of stability and retention of prostheses cause a reduction in the quality of life and satisfaction of these patients.[21–25] Compared to fixed complete dentures, overdentures are a better option due to their lower cost, relative simplicity, and providing the opportunity to remove the prosthetic device for cleaning by a third party, especially for patients with motor or cognitive problems.[15,26] In line with these advantages, overdentures are indicated for patients diagnosed with orofacial digital syndrome (OFD), such as Papillon–Leage–Psaume, as they have cleft palates in up to 50% of individuals affected by the disease and the most common dental anomaly is the absence of teeth.[27,28]

Thus, using overdentures as a form of rehabilitation in patients can lead to a significant improvement in their quality of life from a psychological point of view due to the presence of teeth in patients with agenesis and improved aesthetics in patients with malformations or the absence of teeth. From a nutritional point of view, overdentures can improve masticatory functions leading to the ability to eat harder foods such as vegetables. In regards to oral health, overdentures provide temporary closure of cracks during feeding by the prosthesis, as well as the ability to remove the appliance to perform oral cavity and appliance hygiene to maintain adequate oral health.[29–32]

Overdentures can be supported by implants or the remaining natural teeth. The advantages of preserving and using the remaining teeth include a reduced need for surgeries to install implants, bone grafts due to bone scarcity makes the treatment less costly, in addition to preservation of alveolar bone, the periodontal ligament, proprioception, and good masticatory efficiency.[33–36] However, several factors such as age, bone and mucosal tissue, hygiene, number, and position of remaining teeth must be taken into account when choosing whether the treatment will be performed on the remaining teeth or implants and the retention system.[27] The most important thing to consider is the personalization of the treatment to benefit and prolong the rehabilitation of individual patients as much as possible.

Currently, various types of attachments are used in retention systems.[37–41] The splinting system, for example, uses a bar and clip-type as the group without splinting uses magnetic attachments, a locator, a double crown, and a ball,[42] which is most commonly used.[21]

The advantages of using a ball attachment include ease of hygiene, requires less space than the bar system, lower cost, and simpler technique for installation and use.[43,44] The functional movements of the insert and removal of the prosthesis, in addition to the parafunctional habits, microbiota, and oral cavity, make up the main disadvantages of this system, resulting in loss of retention over time and the need for frequent replacement and maintenance,[24,45–48] mainly in implants that are not parallel.[44]

However, the anatomical limitations, bone quality of the patient, and the technical experience of the dentist can reduce the parallelism of the implants. Implants in unfavorable positions generate lateral forces that, in excess, can cause early failure of the implants, sharp wear, and fracture of prosthetic components.[43,44]

Other factors can lead to wear and deformation of attachments, such as overdenture instability, diet, muscle strength, and high concentrations of stress at the ball attachment as a result of stress that comes from functional or parafunctional habits. This load can increase the wear speed of the O-rings and, consequently, retention loss.[49] In addition, it is noteworthy that patients with parafunctional habits may present with temporomandibular disorders (TMD) involving the stomatognathic system, especially the masticatory muscles and the temporomandibular joint (TMJ), which can generate pain, restriction of movement, and consequent decrease in the quality of life of these patients.[50–53] Joint inflammation of the synovial membrane is responsible for the pain, which is caused by excessive use of force within the TMJ. This can cause stretching and twisting of the joint capsule, surrounding disc tissue, and ligament, as well as osteoarthritis.[53]

In addition, loss of ball attachment retention can occur due to differences in the surface and strength of the different materials used, such as metal, nylon, and plastic. The different properties of these surfaces promote quicker wear of the components as well as a reduction in their resistance.[18,54] Deformation, wear, and degradation increase the internal diameter of the O-rings leading to a loss in retention. This is mainly due to a lack of contact between the parts, which reduces the coefficient of friction, and consequently, the O-ring slides and comes off with a lower holding force.[46,55]

The study of a material's properties is fundamental to understanding how wear occurs and causes a loss or increase in fatigue resistance and retention of the ball attachments and their O-rings. The literature also contains gaps in the study of the physical, chemical, and biological properties of these materials. Most studies cite the loss of retention as a consequence of deformation. However, few studies specifically mention how the process develops and how to avoid it, this gap in knowledge fails to provide a clinically meaningful analysis of dental materials. Through clarification of these answers, we hope to find a solution to the main problem experienced when using this type of attachment, which is the loss of retention.

Thus, studies on the fatigue of ball attachments and their O-rings are necessary for the researcher and clinician to be able to rely on results based on scientific evidence when selecting the correct attachment. In addition, understanding a material's main disadvantages, seeking to reduce them, and exploring alternatives and solutions benefit patient care which highlights the importance of this research.

This study aimed to evaluate the fatigue resistance of ball attachments and their components, such as O-rings and capsules, through a systematic review. Also, we tried to answer the question: "What are the reasons fatigue of the ball attachments occurs?" To help researchers and dentists select or make the ideal prosthetic component based on physical, chemical, mechanical, and biological properties to enhance durability and prolong the treatment time of patients with supported overdenture implants.

Methods

Protocol and registration

This research was conducted and structured in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses protocol (PRISMA),[56] and was registered in the Open Science Framework (osf.io/6839h).

Eligibility criteria

The criteria for this review were made using the PICOS framework: **P**opulation – ball attachments and their components; **I**ntervention – fatigue of the attachment; **C**omparison – different models of ball attachments and their components untested and tested; **O**utcomes – reasons leading to fatigue; and **S**tudy design: in vitro studies. The question answered with this research is: "What are the reasons fatigue of the ball attachments occurs?"

As inclusion criteria, only articles that contemplated ball attachments and methods that used O-rings or their respective capsules were selected. It included tests on fatigue, published in English from 2000 to 2020, and only research articles were considered. The exclusion criteria were articles that did not include ball attachments, especially in relation to their prosthetic components, such as O-rings and capsules, and that did not include fatigue retention tests.

Information and search strategy

An electronic search was performed using the Cochrane Library, LILACS, PubMed, ScienceDirect, and Web of Science databases with the terms "overdenture" AND "ring" AND "fatigue", "overdenture" AND "attachments" AND "fatigue", "overdenture" AND "retention system" AND "fatigue", and "overdenture" AND "abutments" AND "fatigue" to find articles whose main theme was evaluating causes of fatigue for ball attachments used in overdentures.

The searches included in this study were carried out manually by the researchers. A reference management program (Mendeley Desktop, London, UK) was used to remove duplicate references to facilitate the reading and final selection of articles.

Study selection

This research was conducted in two principal phases. In the first step, two reviewers independently, (M.R.C) and (A.L.B), screened the titles and abstracts to identify eligible studies. The second step after the eligibility studies was identified required all collaborators to read the full texts that met the inclusion criteria. With the potential studies identified, the two reviewers discussed with the coordinator (A.C.R) to decide if the studies were to be included in this review.

Data collection process

The data extracted from the papers are in Table 1, which includes the authors, year of publication, aim, number of samples, number of cycles, and the results of the fatigue tests.

Table 1. Main results of the in vitro studies

Author	Aim	Population size	Cycles/pulls	Results
Agrawal, 2017[2]	Determine fatigue resistance with 2 methods of capture (Direct and Indirect)	3	4.320 cycles	Higher retention with direct capture of the ball attachment
Aroso, 2016[7]	Compare durability and retention with 0, 10 and 20°	9	5.400 cycles	When angles increase, the retention tends to decrease
Bayer, 2009[26]	Evaluate the retention force changes	10	10.000 cycles	The componente with gold matrix showed better stability of retention
Botega, 2004[10]	Evaluate retention force and fatigue resistance	5	5.500 cycles	Nitrile rubber showed stable retention when testeds
Branchi, 2010[21]	Evaluate in vitro the retention force and wear resistance	5	5.500 cycles	Materials with noble alloys and Teflon showed increase in the retention
Chaves, 2016[13]	Evaluated the simulation retention force of o'rings used for mini-implant	10	540 cycles	Retention 24% lower after simulation of 6 months
Choi, 2017[5]	Evaluate different angulations (0, 15 and 30°) on the retentive characteristics	10	1.080 cycles	Significant decrease in retention of the o'ring was showed at all angulations
Fatalla, 2012[20]	Evaluate the retention force and fatigue	5	2.880 cycles	Decrease retention after all cycles
Frasca, 2014[23]	Compare retention forces	4	3.240 cycles	Metal material of the capsule presented higher retention values
Galo Silva, 2019[19]	Evaluate psychal-mechanical and morphological properties of polymeric materials	20	2.900 cycles	PET and Polyacetal showed better retention values
Marin, 2018[3]	Evaluate the retention force and wear characteristics	10	5.500 cycles	Polyamide demonstrated increase without damage in the nitrile ring, but increased roughness, wear and deformation in matrix of the mini ball
Mariotto, 2020[22]	Evaluate effects of different cleansing solutions on the psychal-mechanical properties	60	2.900 cycles	The PET material showed the better retention values
Memarian, 2018[12]	Compare retentive values after fatigue testing	5	5.500 cycles	Decrease retention after all cycles
Reda, 2016[4]	Compare retention values	10	5.500 cycles	Retention values decrease after all cycles
Shastry, 2016[15]	Compare the change in the retentive force and removal torque	5	5.000 cycles	Capsule with PET material had increase retention
Sultana, 2017[9]	The effect of implant angulation (0 and 20°) on the retention	7	10.000 cycles	Retention loss greater in the first 500 cycles
Valente, 2019[6]	Develop a new attachment system for overdentures with polymeric materials	20	3.625 cycles	Polyacetal material presents higher retention and metal showed a lowest deformation
Yang, 2011[8]	Evaluate Retention and lateral force with 0, 15, 30 and 45°	1	10 cycles	When angles increase, the retention tends to decrease

Assessment of the risk of bias

The risk of bias was classified as low risk when the article was very clear and anything could alter the results, unclear when we needed to justify if this was valid for the article, or high risk when there was a serious risk of altered results. The articles were classified according to the methodological quality of included studies by the Joanna Briggs Institute.[57]

Results

Study selection and characteristics

The search for articles is detailed in the PRISMA diagram model (Fig. 1). The databases identified a total of 905 articles, of which 367 were excluded as they were from book chapters and annual conferences. Thus, 538 articles were left to read the titles and abstracts. of these, 40 articles were selected. of the 40 remaining articles, duplicates were excluded, and only 23 articles remained. The remaining 23 articles were read in full, and those that did not meet the inclusion criteria were excluded, which resulted in the selection of 18 relevant articles.

Risk of bias

The risk of bias is analyzed following the Joanna Briggs Institute Critical Appraisal Checklist for Quasi-Experimental Studies. Seven questions were analyzed using the answer: high risk, unclear, or low risk (Fig. 2 and Fig. 3). The questions consisted of the following: Is it clear in the study what is the 'cause' and what is the 'effect'? Were the participants included in any similar comparisons? Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest? Was follow-up complete and if not,

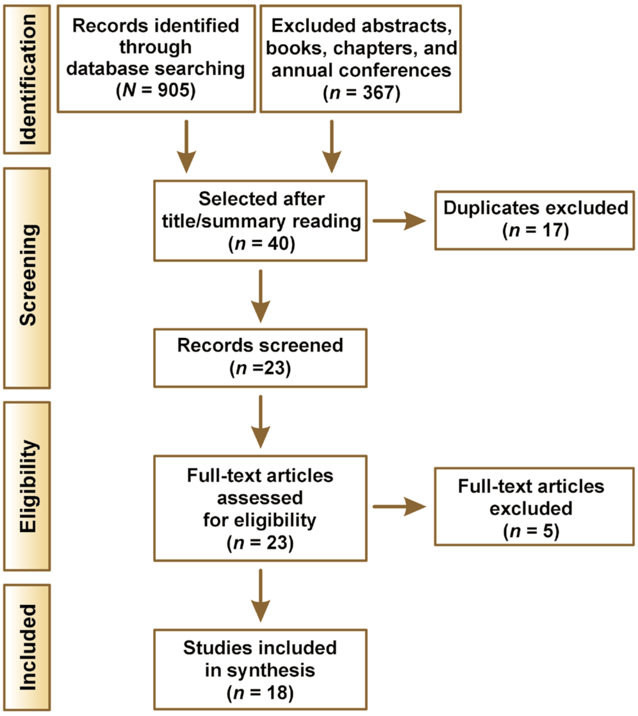

Fig. 1. PRISMA flowchart

were differences between the groups in terms of their follow-up adequately described and analyzed? Were the outcomes of participants included in any comparisons measured in the same way? Were outcomes measured in a reliable way? Was appropriate statistical analysis used?

The principal high risk of bias found in 8 studies was for the question: "Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?" This is due to the different angles used to determine fatigue resistance in the articles of Aroso et al.,[42] Choi et al.,[23] Sultana et al.,[44] and Yang et al.,[43] and also for the thermocycling analysis made by Agrawal et al.,[18] Galo Silva et al.,[58] and for the Marioto et al.,[59] because they used cleansing solutions to evaluate the fatigue. These are facts that can alter the results in comparison to other studies. One study showed "unclear risk" due to the method used to evaluate the results of the retention. Shastry et al.[55] made 100 pulls for the results rather than the cycles that simulate the months like the other studies.

Results of individual studies

The matrix that had the largest decrease in retention in seven studies after the fatigue tests were conventional O-rings.[22,47,48,54,58,60,61] One study demonstrated that the ball system presents higher fatigue retention values when the direct capture technique of the O-ring was performed compared with the indirect technique.[18] Two studies demonstrated that O-rings made of nitrile had stable retention under cycles.[21,45]

Three studies presented matrices with different polymers, such as teflon, polyacetal, polytetrafluoroethylene (PTFE), polyethylene terephthalate (PET), and polyethylene. Three of them – PET, polyacetal and teflon – presented better results with higher retention values.[24,58,61] One study demonstrated that PET had increased retention through immersion time in distilled water, alkaline peroxide, and sodium hypochlorite.[59]

One study compared plastic against a metal capsule. The metal material had higher retention and no significant retention loss over time.[62] Another study demonstrated that noble alloys displayed an additional retention of 50% in the first 250 cycles, with a small increase until cycle 5,500.[61] However, in three different matrixes made of gold, one study showed that the retention values were stable or slightly decreased, but not increased.[55] Two studies showed a decrease in retention using titanium material.[61] Three studies with angled implants in the fatigue tests showed that with increases in the angle, the decrease in retention is greater.[42,43,44]

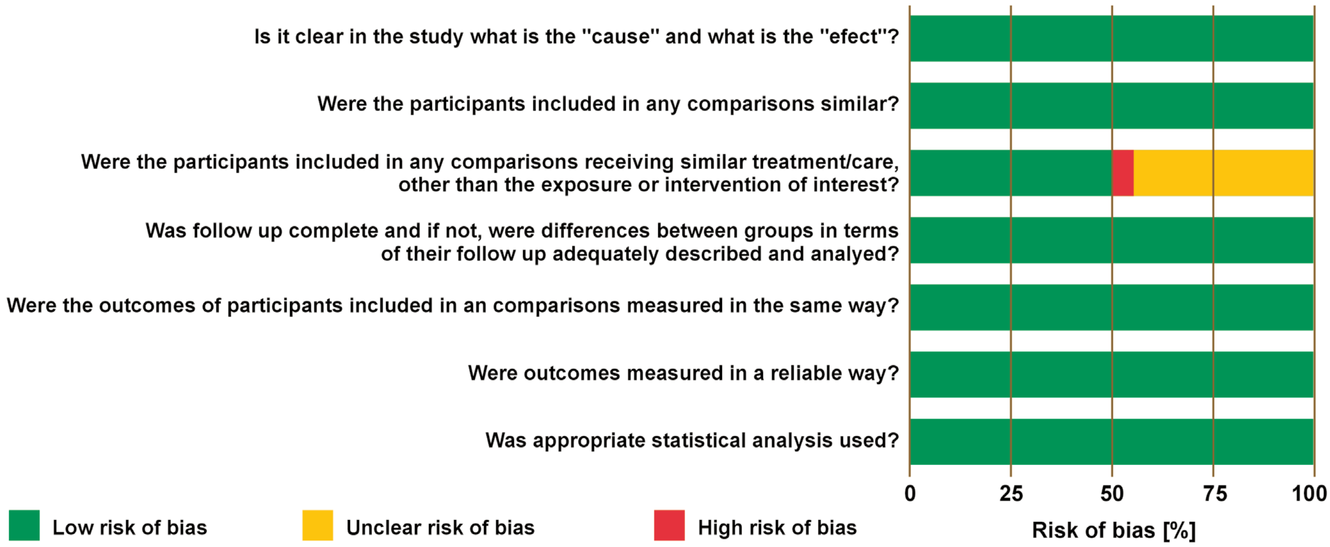

Fig. 2. Risk of bias graph

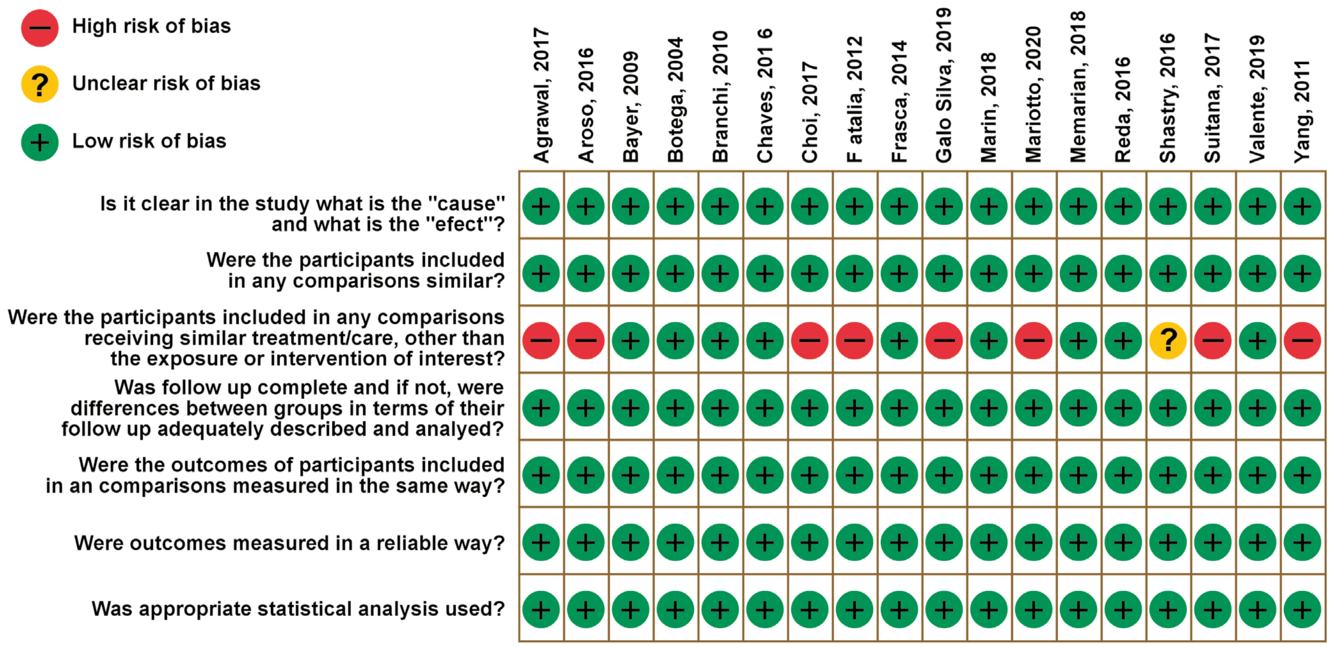

Fig. 3. Risk of bias summary

Discussion

A limitation of this review was that only in vitro research was used, this fact is because it's hard to analyze material properties. Due to the heterogeneity of the studies included, it was not possible to perform a meta-analysis. However, all articles included in this systematic review presented analysis and results about the fatigue and retention values of the ball attachment and their components.

Considering cases where all factors are equal, the choice of attachment should be ideally selected based on its composition. Thus, for the selection of the best materials for making components, fatigue resistance becomes an important analysis since it demonstrates which materials can be used for this purpose, clarifying the physical, chemical, mechanical, and biological properties of these, so that the goal is the long-term durability of the treatments.

Allied with the materials, the prostheses must be built in an ideal occlusal-centric relationship, so that the positioning of the condyles is always correct. This avoids an even earlier wear of the prosthetic components caused by a poor occlusal relationship and TMD caused by trauma, parafunctional habits, stress, muscle dysfunction, and bone morphological changes that cause symptoms such as muscle spasms, pain, and changes in joint position and disc displacement due to mandibular malposition.[53,63] Special attention should be given to patients who have already suffered fractures of the mandible, such as the condyle, because they may present facial asymmetry and malposition of the condyle when in contact with the mandibular fossa if they are not treated properly.[63] Thus, building the complete denture or overdenture within the correct pattern of centric and occlusal relationships is essential. This allows the homeostasis of the region to be maintained and the rehabilitation treatment to have a good prognosis that does not damage the structures, which will act on different planes.

Treatment with overdentures, whether on teeth or implants, is important to rehabilitate patients. They are beneficial in patients whether they are partial or totally edentulous patients, patients with syndromes such as OFD, which is characterized by the presence of a cleft palate with hypoplastic or even total or partial absences, or patients with TMD such as disc displacement.[27,28] The overdentures, in these cases, can act as a therapeutic treatment, acting to stabilize the disc, close the cleft palate, and improve facial aesthetics and occlusal stability.[63] However, special attention must be given to this treatment modality, mainly due to the large number of procedures that are necessary in cases with OFD syndromes, therefore, there must be a very cohesive integration between dental specialties such as endodontics, surgery maxillofacial, prosthesis, and implantology, to achieve effective treatment and prevent early failure of the attachment/prosthesis system.[27]

With the passage of time, deformation and, therefore, a decrease in the retention of most attachments leads to treatment failure.[64] Several studies with parallel implants, angled implants, and innovative capsules were inserted into this systematic review to see how each material reacts to fatigue testing.

The literature reports that values between 5 N and 7 N are sufficient to retain and stabilize an overdenture prosthesis in position during its use.[65] Several materials are used to manufacture ball attachments, and the change in material alters the results of the tests, which have different properties.[18] In addition, the study method, using parallel implants or not, also produces different results. These differences made it impossible to prepare a meta-analysis, on the other hand, the heterogeneity of the studies adds value to the discussion of research and provides us with important information about different materials used for ball attachments.

Different materials were used for ball attachments,[21,45,61,62] which is favorable for discussion since there are numerous O-rings and capsules available on the market. The O-ring is usually composed of nitrile, characterized by its resistance to wear, compression, and corrosion which is the principal factor in the stable retention of this attachment.[60] There are also ball attachments made only of metal, which gives greater retention to the system. Other materials that are stable under fatigue resistance are metal systems that use a gold alloy for the retaining component. This alloy presents ductility and malleability that will promote adaptation and maintenance of the contour, which confers retention stability.[45,66] However, its main disadvantage is its high cost, which makes treatment with overdentures more expensive, which takes away one of its main advantages compared to fixed prostheses, for example.[45,66]

Polyamide compound O-rings have also been evaluated in this systematic review. These showed a higher retention result than conventional O-rings made of nitrile. This is because polyamide has characteristics such as chemical impact and abrasion resistance, besides offering flexibility, temperature resistance, and dimensional stability. These characteristics can be used to explain the greater stiffness of this type of O-ring.[21] Furthermore, matrix and patrix dimensions also influence attachment retention. Larger patrices present higher retention values compared to attachments with smaller dimensions, using the same matrix for both.[45]

According to several studies that analyze fatigue resistance, it has been demonstrated that with an increase in the number of cycles in the trials, retention tends to decrease in the vast majority of ball attachments, as demonstrated by Fatalla et al.,[60] Chaves et al.,[48] Reda et al,[22] and Memarian et al.[47] This fact can be explained by the increase in surface roughness, which can cause plastic deformation of components as the number of cycles increases and consequently there is a reduction in retention.[48,67] Furthermore, this decrease may be due to the different compositions of the plastic materials, as well as the size and shape of the capsules used. Another fact to be considered is the presence of micro-movements, especially if we use parts of different brands in the same retention system.[47,68]

Since most current ball attachments suffer from loss of retention, new studies have shown that new attachment designs are made to solve the main problems related to them. As demonstrated by Valente et al.[24] and Galo Silva et al.,[58] capsules composed of Polyacetal and PET presented with higher retention than the commercial metal O-ring used in their studies. This fact can be attributed to the increase in the roughness and hardness of polymeric materials, as well as the thermal expansion that may have occurred during the experiment, in addition to the design and intrinsic properties of polymers.[24,58] Complementing this analysis, Galo Silva et al.[58] and Shastry et al.[54] performed thermocycling after fatigue tests and obtained different results, where the capsule made with PET showed an increase in retention, probably due to its thermal stability property.[69]

It is known that all prostheses on implants should be sanitized so that the treatment is effective and does not have periodontal and peri-implant problems or even loss of dental implants since bacteria can adhere to these materials.[70,71] There is also the possibility that bacteria such as *Candida albicans*, *Streptococcus mutans*, and *Staphylococcus aureus* present in the oral cavity can cause severe diseases, such as bacterial endocarditis, aspiration pneumonia, or generalized infections of the respiratory tract.[72–75] The cleaning and ability to remove the overdenture is an advantage, especially for patients with syndromes, cognitive problems, and patients who have difficulty opening their mouths due to problems such as hyperplasia of the coronoid process, which makes adequate oral hygiene of the prosthetic device unfeasible.[27,28,76] However, these materials may cause changes in prosthetic components, such as loss of retention, retention gain, or even no alteration, as demonstrated by Mariotto et al., who submitted retention capsules made of polymeric materials in sanitizing solutions and fatigue tests.[59] The results obtained are because of the intrinsic characteristics of these materials, which can be hardened with repetitions and solutions, increasing their retention, as well as chemical resistance which will prevent a loss or gain of retention.[77]

The present systematic review aimed to demonstrate the main reasons that lead to ball attachments and their components to fatigue and treatment failure. Several factors must be taken into account, especially the understanding of the mechanical, chemical, and biological properties of materials, which will be in continuous contact with different temperatures, substances, and forces. Allied with these factors, there is the correct choice of treatment, whether it will be performed with dental implants or natural teeth. This must be guided by strict criteria, observing the different problems that patients may be associated with, such as syndromes, dysfunctions, degree of hygiene, and motor dexterity, so that the treatment has an effective prognosis, increases the quality of life, preserving or restoring health, and has a cost compatible with the patient's wishes and possibilities. In any case, overdentures and their attachments present a significant improvement in quality of life, aesthetics, masticatory efficiency, retention, and stability, which makes them an excellent rehabilitation option.

Conclusions

The main factor to be considered about fatigue resistance is the retention of these components and their low durability. The loss of retention is due largely to reasons such as the materials that compose the attachments and O-rings, size, angulation of the implants, and time of use of the prosthesis. The mechanical properties had a greater impact on the retention stability of attachments. Thus, the answer to these questions is only possible provided that further research is carried out to understand the biomechanical behavior of the materials and new components to be proposed to provide shorter clinical time spent by the professional with regard to maintenance and that the patient has a lower cost of treatment.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets and all supplementary materials 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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Review

Platelet-rich plasma and platelet-rich fibrin in oral surgery: A narrative review

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Abstract

Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) are biological products derived from the plasma fraction of autologous blood that have a platelet concentration above that of the original blood. Cytokines and growth factors are present in platelet-based preparations, and their application has gained great attention in dentistry. The aim of this review was to comprehensively examine the latest scientific evidence on the use of PRF and PRP in oral surgery, and to describe current operational protocols. Platelet-rich fibrin is used after third molar extractions, in the treatment of alveolar osteitis and trismus, and in implant surgery. Platelet-rich plasma is utilized in sinus lift procedures, after tooth extractions, and in patients undergoing the treatment of bisphosphonate-related osteonecrosis of the jaw. Based on this review, plenty of data indicates that the PRF-PRP usage in oral surgery shows promising results. However, no consistent protocols have been presented in the analyzed articles. Further research is needed to provide clinicians with evidence-based clinical recommendations and to develop protocols on the use of these preparations in dental surgery.

Keywords: dental surgery, platelet-rich plasma, platelet-rich fibrin, operative protocols

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Introduction

In terms of the general use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in the field of dentistry, these biological products are widely used in oral surgery.[1–3] The potential of these biomaterials in dentistry is based on the presence of cytokines and growth factors in platelet-based preparations that support the healing process.[1,4] There is, however, no consistency in the laboratory preparation of these materials, and clinical protocols for the use of PRP and PRF in dental patients are diverse (Fig. 1).[3]

The aim of this article is to present the latest reports on the use of PRF in oral surgery, which is currently a subject of interest to the medical community. In recent years, many questions have been asked about the relevance of using PRF in several dental surgical procedures, including third molar extractions, dental implantology, and in patients undergoing bisphosphonate (BP) therapy. This review intends to synthesize the current knowledge and show the possible advantages and limitations of the use of PRF during surgical procedures in the maxillofacial region.

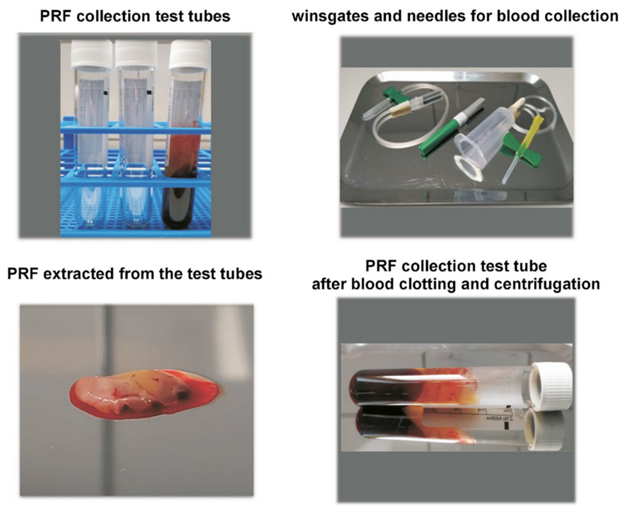

Fig. 1. Laboratory preparation of platelet-rich fibrin (PRF)

Methods

PubMed and Google Scholar were searched using the terms “platelet-rich plasma”, “platelet-rich fibrin”, “PRF”, “PRP”, AND “oral surgery”. The abstracts resulting from the searches underwent an initial review to see whether they fit with the search requirements. Articles were selected based upon their relevancy to general medicine, prioritizing original works, case reports, clinical trials, and clinical practice guidelines based upon the quality of the journal and the authors’ experience in the area.

Types of platelet concentrates

In 2009, Dohan et al. proposed a classification system for platelet concentrates (PCs) based on the method of preparation, differences in content, and the properties of the received fibrin network.[5] The authors distinguished four groups of PCs:

– pure PRP/leukocyte-poor PRP (PPP/P-PRP);
– leucocyte and PRP (L-PRP);
– pure PRF/leucocyte-poor PRF (P-PRF); and
– leucocyte and PRF (L-PRF).

The properties of each concentrate are dependent on the preparation technique and are presented in Table 1. Table 2 presents the comparison between some clinical and laboratory parameters used in surgery research with PRF.

Use of PRF after mandibular third molar surgery

Removal of the third molars is one of the most frequently performed procedures in oral surgery.[1,4] PRF is widely used to facilitate the healing process after tooth extraction, as postoperative wound healing is a multi-stage complex process that aims to re-establish tissue integrity and functional efficiency.[6] Although PRF is commonly used after

Table 1. Properties of particular platelet concentrates (PCs)

PC Type	Protocol	Method	Anticoagulant	Centrifugion	Time	Cost	Platelet content	Leucocyte content	Fibrin density
P-PRP	automated	Vivostat[4]	+	heavy	long	expensive	++	–	low
		Cell separator PRP[5]	+	heavy	very long	very expensive	+++	–	low
	manual	Anuita’s PRGF[6]	+	heavy/light	long	cheap	+	–	low
		Nahita PRP[7]	+	heavy/light	long	cheap	+	–	low
L-PRP	automated	PCCS PRP[3]	+	heavy	long	expensive	++	+	low
		Magellan PRP[8]	+	heavy	long	expensive	++	+	low
	manual	Ace PRP[7]	+	heavy/light	long	expensive	++	+	low
		Cursan PRP[5]	+	heavy/light	long	expensive	++	+	low
P-PRF	manual	Fibrinet PRFM[4]	+	heavy/light	long	expensive	++	–	high
L-PRF	manual	Choukroun’s PRF[9–11]	–	light	short	very cheap	+++	+	high

P-PRP – pure platelet-rich plasma (PRP)/leukocyte-poor PRP; L-PRP – leucocyte- and platelet-rich plasma; P-PRF – pure platelet-rich fibrin (PRF)/leukocyte-poor PRF; L-PRF – leucocyte- and platelet-rich fibrin.

third molar extractions, the outcomes in terms of post-surgical difficulties remain unknown, and randomized controlled trials to vindicate its use are lacking.[1,7–9]

Influence of PRF on postoperative pain and swelling

In a study conducted by Singh et al., 20 patients underwent bilateral extraction of the third molars followed by the application of PRF into only one extraction pocket (study site), while the other site was left untreated (control site).[10] The results showed that there was less pain and that soft tissue healing was better in the PRF pocket.[10]

Ozgul et al.[11] performed a study on 56 patients after bilateral third molar extractions, followed by the application of PRF to only one pocket (the same process as in the study performed by Singh et al. cited above).[10] It was reported that, on the first and third days post-surgery, horizontal swelling, measured from the tragus to the commissure, was significantly decreased. However, in the work of Gülşen and Şentürk performed on 30 patients, the results showed no statistically significant difference in post-surgical pain between the study and the control groups.[12]

Trybek et al. performed a study involving 90 patients with impacted lower third molars.[13] All surgical procedures were performed under an antibiotic cover of 600 mg clindamycin administered one hour before the surgery. The results showed that patients from the study group (with PRF application in the extraction pocket) reported a lower pain intensity at 6 hours, 1 day, and 3 days after surgery. In addition, body temperature was significantly higher in the control group than in the study group on day 2 postoperatively. The authors also observed that trismus was significantly lower in the study group than in the control group at 1, 2, and 7 days after surgery. However, PRF application did not significantly affect the intensity of swelling. The authors claimed that the application of PRF may lead to less traumatic treatment and faster recovery.

Impact of PRF on alveolar osteitis

Alveolar osteitis (AO) is observed in 0.5–5% of patients after routine dental extractions.[14] However, the incidence of AO following the extraction of mandibular third molars is reported to range from 3.9–29.6%.[15,16]

Yüce and Kümerik conducted a study of 40 patients with positively diagnosed and untreated AO within 3 days after extraction of a mandibular third molar.[15] The participants were divided into two groups of 20 patients. The patients from the control group had their extraction pockets curetted and rinsed with saline, while the patients from the study group underwent the same procedure and had PRF applied to the pockets. All patients were examined on days 1, 3, 7, and 15, and 1, 2, and 3 months after the procedure. The soft tissue healing process was evaluated postoperatively using the Wound Healing Index of Landry (Turnbull and Howley). There was a statistically significant difference in the timing of the epithelialization process observed between the groups. The healing rates were significantly faster in the PRF group compared to the control group on every day of examination. Pain was evaluated using the Visual Analogue Scale, and it was found that the pain scores in the study group on the first, third, fifth, and seventh postoperative days were significantly lower than in the control group. It was concluded that the application of PRF might improve and accelerate the therapeutic process of tissue regeneration and may have a positive effect on pain reduction in the management of AO. In addition, the trials conducted by Eshghpour et al.[17] and Al-Hamed et al.[18] both confirmed a significant decrease in the occurrence of AO after the application of PRF to the extraction pocket.

Osteoblastic activity after PRF application

Currently, two studies have evaluated the effects of PRF on osteoblast activity.[1] According to Baslarli et al.[19] and Gürbüzer et al.,[20] there were no statistically significant differences in the activity of osteoblasts between the case (extractions followed by application of PRF) and the control groups (traditional extractions). In both studies, the results were estimated using bone scintigraphy based on the uptake of technetium-99m methylene diphosphonate in the extraction pocket.

Effect of PRF use on trismus

Trismus is a spasm that is a frequent problem in oral and maxillofacial surgical practice. The causes of this condition may be generally classified as articular or extra-articular. Shires et al. have defined trismus as a lengthened tetanic spasm of the masticatory muscles, which restricts mouth opening.[21,22] However, it is often used as a synonym for a decreased range of mouth opening ascribed to extra-articular causes.[23] Trismus is one of the most common complications that occur after removal of mandibular third molars.[24,25]

Uyanik et al.[26] initially performed 40 extractions of impacted mandibular third molars in 20 patients, and repeated their study in 2016 on a group of 56 patients (21 bilateral extractions and 38 unilateral extractions).[27] Both studies showed statistically significant differences in the extent of trismus between the study group (extractions followed by application of the PRF) and the control group (traditional extractions), only on day 1 after the surgery. They did not observe any statistically significant differences between groups on later days.

In sum, recent studies have shown a positive effect of PRF in reducing postoperative pain and swelling, and decreasing the incidence of AO. However, the beneficial effects of PRF on osteoblastic activity, trismus, and soft tissue healing have not been clearly demonstrated and require further research.[1,7]

Table 2. Comparison between some clinical and laboratory parameters used in surgery research with platelet-rich fibrin (PRF)

PRF impact on:	Reference	Number of patients	Diagnosis at the beginning of the treatment	Technique performed	Disinfectants used before the surgery	Clinical outcome
Alveolar osteitis (AO)	2014 Eshghpour et al.[17]	78 study group: 78 control group: 78 (156 impacted third molars)	bilaterally impacted lower third molars	10 mL of venous blood centrifuged at 3,000 rpm for 10 min surgical extractions in patients with bilaterally impacted lower third molars with the PRF application into one of the two pockets	povidone iodine applied in the oral cavity, pockets irrigated with 100 mL normal saline solution	a significant decrease in the occurrence of AO (OD = 0.44; $p < 0.05$)
	2019 Yüce and Kümerik[7]	40 study group: 20 control group: 20	untreated AO at 3 days after extractions	9-mL blood sample centrifuged at 1,300 rpm for 8 min PRF application into the post-extraction pockets in patients suffering from AO	20 mL 0.9% saline solution into the alveolus	a significant decrease in the pain score in the group of patients with PRF application on the 1st, 5th and 7th post-operative day ($p = 0.000$; $p < 0.05$)
Trismus	2015 Uyanik et al.[26]	20 study group: 20 control group: 20 (40 impacted third molars)	bilaterally impacted lower third molars	10-mL blood sample centrifuged at 3,000 rpm for 10 min traditional extractions in group 1 (n = 10), traditional extractions and PRF application in group 2 (n = 10), piezosurgery and PRF application in group 3 (n = 10), in group 4 (n = 10)	sterile physiological saline solution into the post-extraction residual cavity	a significant difference in the occurrence of trismus on the 1st day: between group 1 – 25.61% and 2 – 9.03% ($p = 0.011$), group 1 – 25.61% and group 3 – 9.3% ($p = 0.019$), group 2 and group 4 – 26.16% ($p = 0.019$), and group 3 and group 4 ($p = 0.043$); no significant differences between the groups on other days ($p > 0.05$)
	2016 Bilginaylar and Uyanik[27]	59 study group: 20 control group: 60 (80 impacted molars, 21 bilateral extractions and 38 unilateral extractions)	bilaterally or unilaterally vertically impacted lower third molars	10-mL blood sample centrifuged at 3,000 rpm for 10 min traditional osteotomies in group 1 (n = 20), traditional osteotomies and PRF application in group 2 (n = 20), piezosurgery in group 3 (n = 20), piezosurgery and PRF application in group 4 (n = 20)	sterile physiological saline solution into the post-extraction residual cavity	no significant differences in the occurrence of trismus between the control group (group 1) and other groups
Immediate implantation	2019 Öncü and Erbeyoğlu[37]	26 study group: 30 control group: 30 (60 immediate implantations)	condition after the extraction and surgical preparation of the implant pocket	9-mL blood sample centrifuged at 2,700 rpm for 12 min extractions of teeth, placing the implant with PRF application into one pocket and without PRF application into the other pocket	saline solution into the alveolus	the stability after 1 week and 1 month was higher for the test group ($p \leq 0.002$); the mean marginal bone resorption difference was significantly lower in the group with the applied PRF ($p \leq 0.05$)
Periimplantitis	2017 Rashmi Shah et al.[22]	1 (5 implants)	<2 mm of attached gingiva exposing 3 implants in the 3rd quadrant and 1 (distal) implant in the 4th quadrant	10-mL blood sample centrifuged at 2,700 rpm for 12 min implantoplasty and the application of 2 PRF membranes to cover 3 implants in the 3rd quadrant; after healing, the same procedure was performed on the other two implants	disinfection of the implant with 0.12% chlorhexidine	neoangiogenesis was observed after 8 days. After 4 weeks, the tissue changed from thin to thick and the acceptable coverage of implant surfaces was noticed
Sinus lift procedure	2012 Tatullo et al.[49]	60 study group: 42 control group: 30 (72 sinus lift procedures)	atrophy of the maxillary bone requiring sinus lift procedure	10-mL blood sample centrifuged at 3,000 rpm for 10 min piezosurgery was performed, and in the lifting procedure, authors used deproteinized bovine bone (Bio-Oss®) itself (control group) or in combination with PRF (study group)	no information	the results suggest that PRF reduces the healing time and accelerates the process of bone cicatrization
	2016 Gurler and Delilbasi[54]	24 study group: 12 control group: 12	<5 mm of alveolar bone in the posterior maxilla	10-mL blood sample centrifuged at 2,700 rpm for 12 min piezosurgery was performed; in the study group, an allogenous bone graft mixed with L-PRF was used as a grafting material, covered with the L-PRF membrane; in the control group, only allogenous bone was used, covered with a resorbable collagen membrane	no information	the results showed slight improvement in post-operative complications in the L-PRF group; however; the differences were not statistically significant ($p > 0.05$)
	2017 Cömert et al.[28]	26 PRP study group: 8 PRF study group: 9 control group: 9	<7 mm residual bone crest height	PRP: 10-mL blood sample centrifuged at 1,000 rpm for 10 min PRF: 10-mL blood sample centrifuged at 3,000 rpm for 10 min modified Caldwell-Luc technique was performed; lifting procedures: cavities were grafted with β-TCP (the control group), P-PRP-mixed β-TCP (the P-PRP group) and PRF-mixed β-TCP (the PRF group)	povidone-iodine solution applied on the skin surface at the perioral region	no statistically significant difference was observed between β-TCP alone and in combination with PRP or PRF ($p > 0.05$); the percentage of new bone formation: 33.40 ±10.43% in the control group, 34.83 ±10.12% in the P-PRP group, and 32.03 ±6.34% in the PRF group

β-TCP – beta-tricalcium phosphate; OD – odds ratio.

Use of PRF in dental implantology

Immediate dental implants

Over the years, immediate implant placement has gained popularity due to its numerous advantages (Fig. 2).[28–30] It has been shown that this method minimizes the period of the treatment and the number of surgical visits, which has a positive effect on the patient's comfort.[31,32] Some concerns have been reported regarding immediate implant placements in the molar area, and it has been suggested that large molar roots may result in an unsatisfactory bone quantity. To overcome this, the standard procedure is guided bone regeneration, which leads to an augmentation of bone around the implant. The literature reports that PRF is a useful source of growth factors, such as bone morphogenetic protein (BMP), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF) , and that it allows the regeneration of adjacent tissues.[28] In addition to this, PRF, being an autologous biomaterial, stimulates cell proliferation, migration and angiogenesis, and prevents infection.[33,34]

After an extraction, the wound area goes through many physiological processes, such as bone resorption and gingival remodeling.[35,36] It has been proposed to place an implant soon after the extraction to protect the osseous complex. Healing of the bone next to the implant starts with the formation of a fibrin clot. The implant surface activates the platelets stuck to the fibrin. Other authors have claimed that platelets are an excellent source of the previously mentioned growth factors, and that the application of PRF leads to better bone regeneration and faster osseointegration of titanium implants.[37,38]

Öncü E et al. studied 26 patients (16 men and 10 women) who underwent 60 immediate implantations (study group: 30 implantations with a PRF clot, control group: 30 implantations without a PRF clot).[37] The procedure consisted of a crestal incision followed by luxation and extraction of the teeth. Following this, the pockets were cleaned, and the granulation tissue was removed. Subsequently, the areas for the implants were prepared, and PRF was applied to one of the implant pockets. In the other pocket, the implant was inserted without a PRF membrane. The flaps were sutured after bringing them back to their original position. Changes in bone loss on periapical radiographs were observed 7 days, and 1, 3, and 12 months after surgery. Resonance frequency was also measured using the Osstell® device, which establishes the stiffness of the bone–implant complex. The implant stability quotient measurements were expressed as numerical values from 1 to 100.[38] The results showed that the stability after 1 week and 1 month was significantly higher for the test group. In addition, the difference in mean marginal bone resorption was significant between groups (0.5–0.7 mm for the test group and 0.6–1.3 mm for the control group).

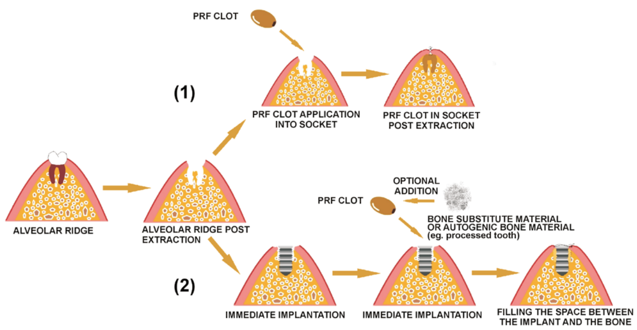

Fig. 2. Applications of platelet-rich fibrin (PRF)

Peri-implantitis

Studies on peri-implantitis have shown that peri-implant complications are not rare, and the fact that an implant survives does not always mean that it was a successful implantation.[39,40] Peri-implant diseases may manifest in two forms: peri-implant mucositis, which is a lesion in the soft tissue around the implant with no signs of bone loss but with bone remodeling, and peri-implantitis, which causes bone loss.[41–43] Various treatment strategies have been proposed for this condition, including pharmaceutical therapy, mechanical debridement, and surgical procedures (e.g., decontamination, smoothing the implant surface, resection, or bone regeneration).[44,45] Modern medicine also allows for the opportunity to use PRF.[46]

Boora et al., in their study on the influence of PRF on peri-implant soft tissue and crestal bone level in a one-stage implant placement procedure, reported heterogeneity in their results.[47] In this study, 20 patients were divided into two groups of 10 people each. The test group had their implants placed with an application of L-PRF, and the control group had theirs placed without it. The participants underwent clinical and radiographic examination at the time of implant placement, and at 1 month and 3 months postoperatively. Less initial marginal bone loss was observed in the test group at the mesial and distal sites of the implants at 1 and 3 months. However, there were no statistically significant differences in pocket probing depth or bleeding on probing between the test and control groups at all time points. The implant survival rate was 100% in both groups.

Hehn et al. examined the effects of PRF on soft-tissue thickening and initial marginal bone loss around implants.[48] This study involved 31 patients, each of whom underwent an implantation procedure in the lower mandible using a split-flap technique. Ten implants were placed with soft tissue augmentation using a L-PRF membrane (test group), and 21 implants were placed without the use of L-PRF (control group). Tissue thickness was measured at the time of implant placement and at 3 months follow-up. Radiographic evaluation was carried out at the time of implant placement, and at 3 and 6 months postoperatively. A statistically significant thickness loss at the crest was observed in the PRF group compared to the control group. There was no statistically significant difference in bone loss observed between the mesial and the distal sides in the control group. The authors concluded that soft tissue augmentation with a L-PRF membrane combined with the split-flap technique cannot be recommended for the thickening of thin mucosa.

Role of PRP in sinus lift procedures

Resorption of the maxilla is becoming a more common clinical condition and requires patient-specific procedures that allow for a reduction in intraoperative timing and maximization of postoperative compliance.[49,50] Focal or generalized atrophy may be caused by multiple factors, but edentulism plays a primary role.[49,51,52]

The idea of maxillary sinus floor augmentation was first described by Tatum (1977) and first published by Boyne and James (1980). In many cases, the procedure is essential to achieve correct placement and positioning of an implant.[53]

In 2012, Tatullo et al. performed a study on 60 patients to investigate, both clinically and histologically, the potential use of PRF combined with deproteinized bovine bone as grafting materials in a sinus lift for severe maxillary atrophy.[49] The results were based on comparisons with a control group, which received only deproteinized bovine bone as a grafting material. In this study, a total of 72 sinus lift procedures were performed. Twelve patients had bilateral atrophy of the maxillary cortex, and the procedures were performed on both of their sinuses, with each of them being applied differently. One side served as the control group and the other as the study group. In addition, patients were divided into three groups according to the length of time between the sinus lift procedure and implant surgery (106 days, 120 days, and 150 days). Before placement of the implant, a transcortical bone sample was obtained from the area of the performed sinus lift procedure, which was subjected to histological and histomorphometric analyses. All treated cases were successful, including both the reconstructive surgery and subsequent rehabilitation with implants. Histological analysis showed that the samples treated with PRF that were collected after 106 days consisted of lamellar bone tissue with interposed stroma that appeared richly vascularized. These results suggest that PRF reduces healing time and accelerates the process of bone cicatrization. According to the authors, it is possible to obtain good stability of endosseous implants placed 106 days after a sinus lift procedure.

A similar study was conducted by Gurler et al.[54] This trial included 28 patients, but data from only 24 were evaluated. The patients were divided into two groups: a control group that had a sinus lift procedure performed using only an allogenous bone graft, and a study group that additionally had a L-PRF membrane used to close a lateral window created during surgery. The results showed a slight improvement in postoperative complications, such as pain, swelling, and the quality and possibility of sleep and eating, in the L-PRF group; although the differences were not statistically significant ($p > 0.05$).

Comparable results were obtained by Cömert et al.[55] and Gassling et al.[53] Both of these studies showed that the use of PRF in sinus lift procedures did not have a statistically significant impact on the success of the surgery, and indicated that further evaluation is needed.

Administration of PRF to patients undergoing bisphosphonate therapy

Bone metabolism disorders, such as osteoporosis and bone metastasis, require therapy with the use of BPs, which are bone-antiresorptive agents.[56,57] Their mechanism of action involves inhibiting osteoclast functions. However, BPs also have a negative influence on fibroblasts and osteoblasts in terms of disabled proliferation.[57–59] There are reports that show a significant correlation between the use of these drugs and an increased risk of osteonecrosis of the jaw, particularly after a local injury (e.g., a tooth extraction).[60–62]

Tooth extraction

Pispero et al. conducted a study in which a 70-year-old woman needed observation of the upper right second premolar (15) tooth due to a suspected fracture.[56] In the medical history, it was reported that she was on an alendronate based therapy for 12 years (one 70 mg tablet per week). The patient went through a professional hygienization a week before the planned surgery. On the day of the extraction, 20 mL of blood was taken from the patient and centrifuged to obtain a PRF clot. Anesthesia and atraumatic extraction of the root of the 15 teeth were carried out. Following this, the pocket was rinsed with a sterile saline solution and closed with two layers of PRF membrane (one in the alveolar pocket and the other above the alveolar pocket). It was decided to continue antibiotic therapy for a further 14 days, along with the application of a 1% chlorhexidine gel to the surgical area three times daily. When the mucosa had regenerated at the end of the second week after the surgery, the sutures were removed. Two months later, at a follow-up visit, no signs of inflammation or exposed bone were reported.

Scoletta et al. carried out a follow-up study that included 63 patients with 202 extractions performed.[63] All of the patients had a history of intravenous therapy with BPs for at least 2 months. Minimally invasive extractions were performed under antibiotic cover (amoxicillin with clavulanic acid 600 mg, 3x per day for 6 days). Post-extraction, the alveolar sockets were cleaned with ultrasonic surgical devices. The pockets were filled with autologous plasma rich in growth factors (PRGF) and sealed with autologous fibrin. At the follow-up, the oral mucosa showed complete healing in most patients (98.41%) and did not differ from that expected in healthy patients. Computed tomographic scans showed normal alveolar bone healing. At the most recent follow-up visit, all patients had unimpaired mucosa and no signs of inflammation. The authors pointed to the significant differences in the duration of the surgical procedures between the present and previous protocols (the previous protocol used a vestibular flap.) Surgical time proved significantly shorter using the current approach.

PRGF may be an important factor in the successful treatment of patients undergoing BP therapy to restore the osteoblast–osteoclast homeostatic cycles via autologous cytokines.[64] Moreover, PRGF can be helpful in shortening the time for recovery from surgical procedures while ensuring good treatment results.[63] It seems that patients who have undergone BP therapy may benefit significantly from using autologous concentrates during surgical procedures, but this subject needs further research and more randomized trials.

Bisphosphonate-related osteonecrosis of the jaw

Kim et al. studied the utility of PRF for the treatment of bisphosphonate-related osteonecrosis of the jaw (BRNOJ).[65] This study included a total of 34 patients. All patients were at first treated conservatively with antibiotics, analgesics, an antibacterial mouth rinse, and daily irrigation with 0.12% chlorhexidine. Surgical protocols included the complete resection of all infected and necrotic tissues, intensive irrigation with antibiotics, the application of L-PRF, and primary closure. The results showed a complete resolution in 26 patients (77%), while 6 (18%) had a delayed resolution, and 2 (6%) showed no response to the treatment. The authors indicated that the treatment of BRONJ with PRF is very promising. However, they emphasized the need for further research using randomized prospective trials.

Gönen et al. performed a study on a 77-year-old male patient who complained about pain and swelling on the left side of his face.[66] In the medical history, it was reported that the patient had prostate cancer and was receiving zoledronic acid for the treatment of secondary hypercalcemia due to the malignancy. Stage 3 BRONJ was diagnosed. Minimal sequestrectomy down to freshly bleeding bone was performed, and a thin layer of necrotic tissue was left to protect the inferior alveolar nerve. Due to the lack of acceptable gingival tissue to close the operative area, a PRF membrane was used to cover the wound. Two layers of PRF were obtained from the patient's blood. In addition, an acellular plasma injection was performed around the wound. In the second week after surgery, epithelialization was observed with no infection or inflammation. In the fourth week postoperatively, new gingival tissue was reported. There was no paresthesia observed. Three months later, total coverage of the bone with new gingiva formation was accomplished. The follow-up lasted for 18 months and no exposure or recurrence was observed. The authors suggested that PRF may be an effective tool for closing exposed bone and promoting tissue healing in patients with BRONJ. However, this subject will need further research on a larger group of patients.

Discussion

PRF and PRP are currently widely used in oral surgery.[1] Third molar surgery is the most common procedure using PRF[1,4,7], and researchers continue to study its influences on postoperative pain and swelling.[13] Recent studies have shown potentially successful outcomes following the use of PRF in extraction pockets, including a reduction in pain and swelling, a lowering of body temperature, and reduced trismus after surgery[10,11,13]; although other clinicians have not observed significant differences.[12] There are also studies confirming that the healing process after PRF application may be faster in patients with AO.[17,67] Significant decreases have also been reported in the extent of trismus after third mandibular molar surgery followed by PRF application[26,27]; although, there are insufficient studies on this topic. It has not been confirmed whether the use of PRF has an impact on osteoblastic activity after extraction.[19,20]

PRF is widely used in the dental implantology field because of its delivery of growth factors that stimulate cell proliferation and angiogenesis.[28,33] It has been shown that PRF application immediately after implantation significantly increases implant stability and decreases bone resorption.[37] There have also been studies on the use of PRF in peri-implantitis treatment, but the results are not consistent and more research on this topic is needed.[47,48]

The application of PRF may be promising in the sinus lift procedure. Studies have shown a reduced healing time, rich vascularization, and faster bone cicatrization.[49] One study has suggested that postoperative pain and swelling may be minimized by using a PRF membrane; however, the observed differences in this study were not statistically significant.[53–55]

There is a significant correlation between using BPs and an increase in the risk of osteonecrosis of the jaw. Researchers are still looking for ways to avoid this complication.[60–62] Several studies have provided encouraging results for the use of PRGF in patients undergoing BP therapy. In the overwhelming majority of cases, proper healing of the mucosa and no bone exposure after dental extraction were observed in the treatment group.[56,63] It has also been demonstrated that this surgical approach may shorten the time for recovery from surgical procedures compared to traditional surgical extraction in patients at risk of BRONJ.[63] Furthermore, the application of PRF may be helpful in the treatment of BRONJ. PRF stimulates the creation of new gingival tissue, decreases the time for healing, and may be a useful tool to help to close the wound after sequestrectomy. However, this topic still requires further studies that incorporate a large group of patients.

Conclusions

Currently, there are many case studies and meta-analyses on the use of PRF in oral surgery. The literature mostly favors the use of PRF and numerous studies have shown promising results. However, this subject needs further research because of the limitations in the previous work. Presently, there are no standard PRF protocols. In order to make the research more reliable, a single standard protocol should be created. It is certain that the successful use of a PRF product depends on the clinician's skill and their understanding of the preparation technology. The growing popularity of centrifuges and the simplicity of the procedures at the dental office present an opportunity for the more widespread use of PRF in oral surgeries.

Ethics approval and consent to participate

Not applicable.

Data availability

All data generated and/or analyzed during this study is included in this published article.

Consent for publication

Not applicable.

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Review

Two years of the COVID-19 pandemic from a child's perspective: A narrative review

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic has been ongoing since 2020. This period is characterized by a significant change in people's lifestyles. Children are a particularly affected group. In order to determine the impact of the pandemic on children's lives, scientific publications available in PubMed, Google Scholar, and The United Nations Children's Fund (UNICEF) Innocenti's Children and COVID-19 Library were reviewed, and the statistical data made available by the Polish Ministry of Health on incidence, death and vaccination rates was analyzed. Even if children were not infected with the virus, they felt the effects of the pandemic through restrictions in the daily functioning of schools, service facilities and households. Despite the relatively mild symptoms accompanying infections in pediatric patients, as well as the low rates of hospitalization and mortality, the pandemic have had numerous negative effects on children's mental and physical health that may trigger further "non-communicable epidemics". Weight changes, limitations in physical activity, and the intensification of social and emotional problems will certainly have a negative impact on their future lives. The introduction of vaccination for children over the age of 5 brought hope, but since then, it has been accompanied by controversy and uncertainty. Further research is necessary to determine the impact of the COVID-19 pandemic on children.

Keywords: vaccination, child, COVID-19, pandemic, SARS-CoV-2

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic is a phenomenon that has changed the functioning of individuals and society, not only in medical but also in economic and social terms. The first cases of this pathogen were reported in November 2019 in Hubei Province, China.[1] Since then, the virus has spread to all continents, resulting in the World Health Organization (WHO) declaring a worldwide pandemic on March 11, 2020.[1] The family of coronaviruses, known to man before the pandemic, was thought to be the cause of seasonal and local epidemics in the Middle East (Middle East respiratory syndrome (MERS)).[2] However, none of the previously reported coronaviruses contributed to a pandemic outbreak on such a large scale.

The pandemic and related restrictions have had a significant impact on people's lives. This is especially true of individuals who are distinct from the general adult population, including children and the elderly. These groups stand out in terms of infectious diseases and public health due to their immunological, cognitive and social differences when compared to young and middle-aged adults. Studies from around the world point to old age as a risk factor for a severe course of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[3] Age >65 years is the most important factor in terms of the severity of the disease, and this is especially true for groups aged >80 years. In addition, male gender, Black and South Asian race, smoking, pregnancy, obesity, and concomitant diseases (e.g., oncological diseases, chronic obstructive pulmonary disease (COPD), heart and kidney failure, hypertension, sickle cell anemia, Down syndrome, or type 2 diabetes) are important risk factors.[3] Respiratory and cardiovascular dysfunction that progresses with age is cited as the cause of this increased risk.[3]

The abovementioned factors, which are specific to the elderly, do not exclude the possibility of a severe course of COVID-19 in the pediatric population. Furthermore, the condition of children is often overlooked in different sorts of research and reports. This is evidenced by the fact that the exact share of children and adolescents suffering from COVID-19 in Poland is unknown.[4] Moreover, it was initially believed that children were immune to SARS-CoV-2 infection and did not manifest any clinical signs of the disease.[5] Although they have relatively milder symptoms of COVID-19 compared to adults, the disease is also a major problem in this age group.[5,6] The most common clinical manifestations among pediatric patients are fever, cough and a runny nose. Gastrointestinal (vomiting and diarrhea), skin (rash) and neurological (headache, seizures) symptoms are also quite common. The clinical manifestation of COVID-19 in children and adults also includes the oral cavity, where blistering, ulceration and exfoliative stomatitis are the most common,[7] with ulcerations primarily affecting the palate and tongue.[7] Fungal infections are also widespread, but in children, the most serious oral complication is Kawasaki-like syndrome.[7] These conditions are mostly due to an exacerbation of the symptoms of pre-COVID-19 diseases, and the COVID-19-specific oral manifestation is a loss of taste and smell.[7]

The disproportionality is also evident in the number of publications on COVID-19. Research papers focusing on adults significantly outnumber publications on adolescents or children. The aim of this study is to analyze the available publications and statistical data on the epidemiology, clinical symptoms and impact of the COVID-19 pandemic on children worldwide and in Poland.

Material and methods

According to the Act of January 6, 2000, from the Ombudsman for Children, a child is a person from conception until reaching the age of majority (18 years in Poland). This definition was adopted in the present paper, excluding intrauterine infections in fetuses.[8] A literature review was conducted using PubMed, Google Scholar, and The United Nations Children's Fund (UNICEF) Innocenti's Children and COVID-19 Library. The following search terms and their combinations, both in English and in Polish, were used when reviewing the aforementioned databases: "COVID-19", "child", "COVID-19 vaccination", "Poland", and "influence." The analyzed publications about COVID-19 were dated from 2020 to 2022, and described different waves of the pandemic, with a focus on the more recent ones. Other articles included in the review were published no earlier than 2015. The review process began on March 15, 2022, and continued for 1 month, until April 2022, with the addition of data from the Polish guidelines published in June 2022.[30]

Initially, the list of titles and abstracts from the available papers was examined, then the authors analyzed the full texts of the chosen publications. Full-text publications from the entire COVID-19 pandemic period were included in the study. Original papers, legal acts, review papers, and case reports were analyzed. No restrictions were applied with respect to the country of origin, but preference was given to publications written by Polish authors. There were also no restrictions regarding the methodology of the analyzed studies. Abstracts, posters and reviews were excluded. A non-systematic approach was used to analyze the included publications.

Statistics published by the Polish Ministry of Health on COVID-19 morbidity, mortality and vaccination rates for this pathogen among children and adults were also reviewed.[43] They were valid for the time when the review was conducted (March and April of 2022). The quoted excerpts of rules and regulations were taken from the Polish Journal of Laws.

Description of the state of knowledge

Epidemiology of infections among children

Children are likely to get infected by SARS-CoV-2 regardless of age.[9] The youngest are particularly vulnerable due to the immaturity of the immune system's defense mechanisms. Maternally-derived immunoglobulin G (IgG) antibodies are decreased in newborns. A lower reactivity of the complement system and a low number of B-cell co-receptors are responsible for a limited humoral immune response. In addition, cells of the immune system, such as T cells, only become immunocompetent when encountering foreign antigens over the years. The immune system does not reach maturity until around 12 years of age.[10]

In the initial phase of the pandemic (until January 30, 2020), out of nearly 25,000 cases reported in Wuhan, China, only 28 (0.11%) were among children.[11] During the following month, this percentage increased to approx. 1.34%. The youngest of the registered cases was a 28-day-old newborn.[11] The report prepared by the WHO indicated that a reliable epidemiological and clinical assessment of the disease among Chinese children was not possible due to the non-specificity of the presented symptoms and the small number of infected minors.[12] It is worth noting that most patients manifested mild cold-like symptoms, mainly cough and fever, that spontaneously resolved within 1–2 weeks. Only 0.20% of children were in critical condition.[12] It has been shown that the most common group among those infected up to 5 years of age were infants (50.0%) of the male sex (53.0%).[13] The presence of viral genetic material has also been documented by the polymerase chain reaction (PCR) testing in newborns (up to 30 h after delivery).[13] In the data from the first wave of the pandemic in England, 1.0% of the infected were children, with an average age of 6 years and the majority of infections observed among infants.[14] The data obtained in American studies was similar – 1.7% of pediatric patients in relation to the total number of infected people.[15] According to later studies, the percentage of juvenile patients fluctuated in the range of 1.0–5.0% in the general patient population.[16]

An increase in the rate of infections in the pediatric population was observed after the emergence of the Delta variant (B. 1.617.2), with a range of 10.0–24.0%.[9,17,18] Compared to the ancestral variants (19A/19B), the Delta variant is characterized by a shorter incubation period (4 days vs 5–6 days), a greater household attack rate (12–17% vs 80–100%) and a larger basic reproduction number (2.7 vs 5–6.5).[18] Currently (as of March, 2022), accumulating data from many countries indicates that this percentage reaches around 16.0%.[9]

Among the reasons for the low morbidity and the mild course of the disease in children is a low expression of renin-angiotensin-aldosterone system (RAAS) receptors (the gateway for SARS-CoV-2 to enter cells), lower angiotensin-converting enzyme activity, and a lower amount of interleukin (IL)-6 and IL-10 in comparison to adults.[16] The sources of infection among children may seem surprising. Children were shown to get infected more often in households from adults than from peers in schools.[17,18] The hospitalization rate remained at a low level (5.0–20.0%), with a significant increase (5–10 times) reported due to infections with the Delta (B.1.617.2) and Omicron (B.1.1.529) variants.[17,19,20] Infants up to 6 months of age were hospitalized most often.[20]

Many researchers, and medical practitioners in particular, point to the problem of underestimation of the data presented and the small number of studies conducted on pediatric patients.[5] Unfortunately, there is no official government data on infections among children in Poland. As of May 15, 2020, during the first wave of the pandemic, out of 17,921 diagnosed COVID-19 cases, only 1,191 (6.7%) of them were children.[21] These were mainly adolescents aged 15–19 years, representing 32.7% of the child population.[21] This result turned out to be surprisingly high, much higher than that reported for other European countries and the USA (0.6–2.0%), and comparable to the data obtained in Canada (5.7%).[21] This is likely due to the large number of tests conducted among children and fewer among adults.

In Poland, the highest proportion of pediatric patients was found in the Silesia Province (20.2%) and the lowest in the Lubuskie Province (3.0%).[21] Studies do not indicate the exact reasons for such a significant difference among provinces, but the authors mention as possible reasons the total number of tests performed, varying epidemic situation and differences in the SARS-CoV-2 transmission (e.g., epidemic outbreaks in healthcare facilities or nursing homes).[21] In September 2020, the proportion of children among those infected reached 7.6%.[22] Until then, no deaths in pediatric patients due to COVID-19 had been reported.[21,22]

COVID-19 symptoms in children

COVID-19 is characterized by a multitude of symptoms. Apart from respiratory tract involvement, the disease may significantly affect other systems (e.g., digestive, nervous, or muscular). In children, the symptoms of the disease are similar to those observed in adults, but they occur with different frequencies.[9] Most children are asymptomatic (15.0–42.0%) or have mild symptoms, to such an extent that children were initially thought not to be potential victims of the virus.[9,23] Most often, the asymptomatic course was observed in children aged 1–10 years (19.7%). Both asymptomatic and symptomatic patients were equally hospitalized.[24] The most common indicators of infection were fever (64.2%), cough (34.8%) and a runny nose (16.1%).[24] Less common symptoms of respiratory tract infection included a sore throat (8.9%), sputum production (2.7%) and dyspnea (10.7%), which were most frequent in infants.[24] Gastrointestinal symptoms included diarrhea (13.4%) and vomiting (6.3%), specific to older children. Headaches affected approx. 5.5% of the examined children.[24] There were also reports of rash (10.7%), conjunctivitis (8.0%), peripheral edema (8.0%), oral lesions (6.3%), cervical lymphadenopathy (2.7%), and even shock (8.9%).[24] Feeding difficulties with accompanying fever were common in infants.[9] In addition, the aforementioned headaches and seizures played a major role among neurological symptoms.[25] The majority of these seizures were febrile seizures, sometimes with electroencephalography (EEG) changes.[26] However, it has not been demonstrated whether these seizures are SARS-CoV-2-induced epilepsy.[27] Non-febrile illness seizures may be immunologically mediated and stimulated by cytokine production.[27] All of them disappeared after appropriate treatment. Neurological symptoms were reported less frequently in children than in adults and were mainly non-specific.[26] Only 1.0% of them, apart from convulsions (0.3%), encephalopathy (0.6%), meningeal symptoms (0.41%), cranial nerve damage, and ataxia, were specific.[26] A loss of smell and taste, often accompanying infections in adults, was less frequent in children (1.0–10.0% of those infected).[9]

The clinical manifestation of COVID-19 in children and adults does not bypass the oral cavity (about 25% of patients), where blistering, ulceration and exfoliative stomatitis are most common,[7] with ulcerations primarily affecting the palate and tongue. They are painful (about 75%) but do not bleed.[7] Fungal infections are also very frequent, arising from stress and lowered immunity. The most serious oral complication is Kawasaki-like syndrome, the symptoms of which include erythema, dryness, cracking, and bleeding.[7] The above conditions are mainly associated with the aggravation of symptoms from pre-infection diseases. The specific symptoms of COVID-19 in the oral cavity are a loss of smell and taste.[7]

In Polish studies analyzing the clinical manifestations of SARS-CoV-2 infection in children in 2020, the most commonly observed symptom was fever (46.0%), mostly lasting for 1–2 days.[28,29] Additional 14.0% of patients had a subfebrile rise in temperature.[28] A loss of smell and taste occurred in only 8.0% of the general pediatric population, in 20.0% of adolescents, and never in those under 5 years of age. An asymptomatic course was found in 21.0% of children. Headache (22.0%) and sore throat (28.0%) were more common among adolescents. Younger children were more likely to have a runny nose, diarrhea, rash, and fever. Radiologically confirmed pneumonia was diagnosed in 12.0% of children. A total of 23.0% of patients presented at least one gastrointestinal symptom.[28] Among the aforementioned conditions, gastrointestinal symptoms, pneumonia and rash were associated with longer hospital stays in children.[29] It is also worth noting that during the second wave of the pandemic (September–December of 2020), there was a lower proportion of asymptomatic patients (13.0%) compared to the first wave (March–August of 2020; 36.0%).[28] However, pneumonia (14.0% vs 9.0%) and gastrointestinal symptoms (29.0% vs 12.0%) were more common.[28] The increase in cases of infected children in Poland in early 2022 due to the Delta and Omicron variants did not worsen the course of the disease, and most cases remained mild or moderate.[30] The most important symptoms of SARS-CoV-2 infection in children are presented in Table 1.

In addition to clinical symptoms, radiological and laboratory abnormalities have also been reported in children with COVID-19.[25] In more than one-third of pediatric patients after the X-ray examination, and in more than half of pediatric patients after the chest computed tomography (CT) examination, the "milk glass" image was seen, most often among adolescents aged 15–18 years.[25] This image was often observed bilaterally, with a predominance in the peripheral regions and lower lobes of the lungs.[28] Echocardiography revealed left ventricular dysfunction in approx. 47.4% of patients, with almost all cases (89.0%) resolving after 7 (5.0–8.3) days.[24] Laboratory tests have also shown significant discrepancies. Some researchers point to an absence of laboratory markers for the disease, with only a slight increase in C-reactive protein (CRP) and procalcitonin levels, while others describe key role for the latter measures and D-dimer levels as predictors of a patient's condition.[9,15,25] A significant percentage of pediatric patients had elevated levels of CRP (54.0%), ferritin (47.0%), lactate dehydrogenase (37.0%), D-dimers (35.0%), and procalcitonin (21.0%), as well as transaminases, creatine kinase MB (CK-MB), and the erythrocyte sedimentation rate (ESR).[31] Patients with pneumonia had higher leukocyte counts and CRP, D-dimer, IL-6, alanine transaminase (ALT), and aspartate transaminase (AST) levels than children without pneumonia.[28] The indicators of the severe course of COVID-19 in children include elevated levels of the abovementioned markers of inflammation, the occurrence of dyspnea with tachypnoea and hypoxia, and alimentary symptoms.[32] Risk factors for such a course and the need for hospitalization are primarily comorbidities, most commonly pulmonary (28.0%), metabolic (i.e., diabetes and obesity; 27.0%), neurological and developmental (22.0%) conditions.[31]

The variety of symptoms and the relatively mild course of infection in children have been common reasons for avoiding COVID-19 diagnosis and underestimating the impact of the infection in pediatric patients. However, concerns regarding the long-term complications of the pandemic in the current pediatric population are understandable.

Table 1. Key demographic aspects, clinical manifestations and effects of the coronavirus disease 2019 (COVID-19) pandemic among children

Topics covered	Study	Main findings
Epidemiology	Jackowska et al. 202[19] Wei et al. 2020[11] Bhuiyan et al. 2021[13]	Children can get COVID-19 regardless of age. Newborns can also get infected.
	Wei et al. 2020[11] Ladhani et al. 2020[14] Alsohime et al. 2020[15] Koirala et al. 2021[17] Howard-Jones et al. 2022[18]	In the first wave of the pandemic worldwide, 0.29–1.7% of infected patients were children; during the Delta variant wave, the incidence rate in pediatric patients increased to 10.0–24.0%.
	Koirala et al. 2021[17] Patel 2020[19] Marks et al. 2022[20]	Hospitalization rate remained at a low level (5.0–20.0%); it increased during the Delta and Omicron variant waves.
	Jackowska et al. 2020[21]	The percentage of infected children in Poland was 6.7–7.6%.
Symptoms	Williams et al. 2020[23]	The most common course was asymptomatic (15.0–42.0%) or mild.
	Yasuhara et al. 2020[24] Paradowska-Stolarz 202[17] Pokorska-Śpiewak et al. 2021[28] Mania et al. 2022[29]	The most common symptoms are fever, cough and a runny nose. Gastrointestinal (diarrhea, vomiting), neurological (convulsions, headache) and dental (blisters, ulcers, inflammation) symptoms have also been observed. A loss of smell and taste was less common compared to the adult population.
	Fernandes et al. 202[32]	Indicators of severe COVID-19 in children include elevated levels of inflammatory markers (CRP, ESR, procalcitonin), the presence of dyspnea with tachypnea and hypoxia, and gastrointestinal symptoms.
PIMS/MIS-C syndrome	Jackowska et al. 202[19] Radia et al. 2021[33] WHO 2020[35]	PIMS/MIS-C is a rare complication, appearing 4–8 weeks after the infection, manifesting with symptoms from the respiratory, cardiovascular, gastrointestinal, and oral systems, fatal in 1.5%.
Effects of the pandemic	Skolmowska et al. 2022[57]	The problem of compulsive overeating and weight gain has increased, especially in obese young women.
	Ezpeleta et al. 2020[58] Kolcakoglu and Yucel 2021[59] Chen et al. 2020[60]	The problem of mental disorders, caused by the occurrence of separation anxiety, uncertainty about the future and lack of the company of peers, has intensified to a mild or moderate degree. It manifested in tantrums, crying and aggression fits.
	Krywult-Albańska and Albański 2021[61]	The problem of deterioration in the quality of education and deepening of technological exclusion due to remote learning was noted, as observed by student's parents.
	Yum et al. 2021[62] Üstün et al. 2021[64]	There is a perceived problem of deteriorating eyesight (more frequent myopia) and reduced frequency of dental check-ups among children.

WHO – World Health Organization; CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; PIMS – pediatric inflammatory multisystem syndrome; MIS-C – multisystem inflammatory syndrome in children.

PIMS/MIS-C syndrome

The multisystem inflammatory syndrome in children associated with COVID-19, known in Europe as pediatric inflammatory multisystem syndrome (PIMS) and in the USA as multisystem inflammatory syndrome in children (MIS-C), is a rare but very serious complication of SARS-CoV-2 infection.[9,33] The criteria for the diagnosis of this syndrome are presented in Table 2.[9,34,35]

Pediatric inflammatory multisystem syndrome occurs approx. 4–8 weeks after the SARS-CoV-2 infection.[9] Its course may resemble Kawasaki disease or toxic shock.[9] Patients diagnosed with this syndrome range in age from 3 months to 20 years, with a median age of 8.6–9.7 years.[33,35] This complication occurs in 1 in 3,000 children infected with the SARS-CoV-2 (approx. 0.03%).[9] Boys of African descent with obesity and respiratory dysfunction are more commonly affected.[33,35] Symptoms in these patients were mainly respiratory (80.0%; cough, dyspnea), cardiovascular (67–82%; hypotension, tachycardia) and gastrointestinal (71.0%; vomiting, diarrhea, abdominal pain). Rash and other skin symptoms were more common than in the course of COVID-19 without this syndrome (42.0%). The Kawasaki-like syndrome is also manifested by a number of symptoms in the oral cavity, including erythema, dryness, fissuring, peeling, cracking, and bleeding of mucosa and the lips, as well as strawberry tongue.[7] In Poland, 8.4% of children with this complication require care in an intensive care unit (ICU), while in other countries this percentage ranges from 40.0% to 60.0%.[9] In laboratory tests, the most common abnormalities were CRP elevated to approx. 150 mg/L (94.0%), neutrophilia (83.0%), lymphopenia (50.0%), elevated troponin T levels (68.0%), elevated N-terminal pro B-type natriuretic pep-

Table 2. Diagnostic criteria for PIMS/MIS-C syndrome according to WHO and Centers for Disease Control and Prevention (CDC)

Source	Description of the criteria
WHO criteria (all 6 must be met)	patient's age: 0–19 years
	fever for at least 3 days
	signs of multi-system activity (at least 2): – rash, bilateral non-suppurative conjunctivitis or symptoms of mucocutaneous inflammation (of the mouth, hands or feet) – hypotension or shock – heart dysfunction, pericarditis, valvulitis or coronary abnormalities (including echocardiograms or elevated troponin/BNP levels) – evidence of coagulopathy (prolonged PT or PTT; elevated D-dimers) – acute gastrointestinal symptoms (diarrhea, vomiting or abdominal pain)
	increased levels of inflammatory markers – CRP, ESR, procalcitonin
	exclusion of another infectious cause of the induced condition
	SARS-CoV-2 infection (positive PCR, antigen test or contact with an infected person)
CDC criteria (all 4 must be met)	age <21
	– documented fever >38.0°C for ≥24 h or reporting a subjective fever lasting ≥24 h – laboratory markers of inflammation
	no alternative diagnoses
	recent (up to 4 weeks) or current SARS-CoV-2 infection (PCR, antigen test, exposure)

BNP – B-type natriuretic peptide; PT – prothrombin time; PTT – partial thromboplastin time; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; PCR – polymerase chain reaction.

tide (NT-proBNP) levels (77.0%), and thrombocytopenia (41.0%).[33,36] Abnormalities were also found in chest radiographs and echocardiography (59.0%), including mainly coronary aneurysms and pericardial effusions (41.0%).[36]

Among hospitalized patients, 1 in 10 required respiratory support in the ICU,[36] and 1.5% of children diagnosed with PIMS died.[32] A registry of patients with PIMS is kept in Poland, and by January 28, 2022, there were 500 children recorded.[37]

Pandemic calendar for children

The COVID-19 pandemic is now more than 2 years old. Like a child, the virus has developed and changed during the first 2 years of life, adapting to environmental conditions. Thus, if one were to plot the natural history of the pandemic on a numbered line, it would resemble a sine wave rather than a straight line.

On March 4, 2020, the first case of SARS-CoV-2 infection in Poland was detected in Zielona Góra.[38] Due to subsequent infections in other parts of the country, on March 10, a decision was taken to cancel all mass events, and a day later, schools, kindergartens and universities were closed until further notice. On March 15, the borders of Poland were closed, and on March 20, it was decided to impose an epidemic state throughout the country.[38] On March 23, the first case of COVID-19 was diagnosed in a Polish child – a 13-year-old girl hospitalized until April 13 in Kraków.[39] The closure of educational institutions was, therefore, one of the first government measures to curb the spread of coronavirus in the country. Schools were only allowed to perform a caretaking function. In the Act of March 2, 2020, on special solutions related to preventing, counteracting and combating COVID-19, other infectious diseases and emergencies caused by them, there was a provision on the possibility of remote work, which opened the way for further decisions concerning children.[40] On March 25, gatherings of more than 2 people were banned. Most institutions and service establishments remained closed. On April 1, a ban on the movement of people under the age of 18 without adult supervision was introduced. Parks, boulevards and beaches were closed.[40]

The first abolition of restrictions happened on April 20. The ban on moving without adult supervision was maintained only for children under 13 years of age. On May 4, nurseries and kindergartens were allowed to return to work under a sanitary regime, in accordance with the guidelines of the Chief Sanitary Inspectorate. On May 30, 2020, most of the restrictions in force were eased, but covering the mouth and nose remained mandatory in public spaces.[38] At that time, the first partially remote school year in Poland had ended. In May 2020, the first cases of PIMS in Poland were reported.

After the return to school, the pandemic situation began to deteriorate rapidly. On October 24, the decision was made to return to distance education for grades IV–VIII of primary school and the entire post-primary school.[38] From November 4, this procedure was extended to grades I–III. On November 28, the outside movement of people under the age of 16 was again restricted. On January 18, 2021, full-time education in grades I–III of primary schools and special schools was restored.[41] On March 25, nurseries and kindergartens were closed, and reopened on April 19. The students were learning remotely practically until the end of the school year. The new school year began in classrooms with a sanitary regime in place. On March 28, 2022, mouth and nose coverings and isolation were abolished.[42]

A large number of rapidly changing decisions issued by the authorities, combined with separation, rich misinformation and a fear of infection, made the COVID-19 pandemic a turning point in children's lives, and caused widespread changes in their health, both mentally and physically.

COVID-19 vaccinations in children

On March 31, 2022, the number of COVID-19 vaccinations administered in Poland was 53,924,321, and the number of fully (2 and more doses) vaccinated people amounted to 22,347,421 (59.0% of the population),[43] with 3.3 million vaccinations given to children aged 5–17 years.[43] These numbers are not optimistic, especially when compared to demographic statistics. The number of children aged 5–18 years in Poland in 2020 was 5,770,408.[44] Taking the uptake of 2 and 3 doses into account, the number of fully vaccinated children represents only a fraction of this population. A disturbing fact is also the lack of widely available vaccinations for children in some highly developed European countries, including Germany and England.

After the development of COVID-19 vaccines for adults, pharmaceutical companies started testing their products for safety in children.[45] These studies showed that the vaccines were safe for the youngest children, and mainly local reactions (pain, swelling, or redness) were reported.[45,46] Myocarditis or pericarditis were reported more frequently in young men than in other groups, but the vast majority of these cases were mild.[46] In addition, the risk of developing myocarditis after SARS-CoV-2 infection was 6–34 times higher than after getting the vaccine.[46] However, it was recognized that immunizing children was essential for a quicker end to the pandemic, and was beneficial for patients in terms of a lower risk of hospitalization and death.[45] Parents and patients themselves were also concerned. According to research results from all over the world, 40.0–70.0% of parents declared a willingness to vaccinate their children (44.0–75.0% in Poland).[47,48] Less enthusiasm was visible among the minors themselves. They expressed a willingness to vaccinate only in 49.6–61.4% of cases.[47] The main concerns were adverse reactions to the vaccines and doubts about their efficacy, often repeated in the media.[47]

Vaccination for those aged 16 years and older was introduced together with the program for adults on December 27, 2020. Teenagers aged 12–15 years old could register for vaccination from June 7, 2021, and children aged 5–11 years from December 14, 2021.[49,50] Currently (as of March, 2022), the following preparations are approved for vaccinating adolescents: Comirnaty by Pfizer (New York, USA)/BioNTech (Mainz, Germany) with a 21-day interval between doses and Spikevax by Moderna (Cambridge, USA) with a 28-day interval.[49] The youngest patients receive Comirnaty with an interval of 3 weeks.[50] It is also possible to be subjected to a three-dose vaccination, with intervals of 21 and 28 days between administrations, intended for children with immunodeficiencies, such as oncological patients, the immunosuppressed, transplant patients, or patients with human immunodeficiency virus (HIV).[50] From January 28, 2022, it is possible for adolescents to receive a booster dose of the Comirnaty vaccine.[51] The doses are not related to body weight and are only adjusted to the age of the vaccinated person. For adolescents, they are the same as the adult dose (30 μg), while children are given a lower dose of 12 μg.[52] This difference is due to the different physiology of the immune system in children and adults.

Arguments in favor of the vaccination of children include protection against infection, reducing the risk of developing PIMS syndrome, and ensuring greater population immunity.[53] Arguments against the vaccination include post-vaccination complications, the relatively mild course of COVID-19 in children and a low hospitalization rate, the limited vaccine supply in less affluent countries, and the higher costs of vaccination programs compared to treating pediatric patients in the ICU.[53] However, it is important to remember that each successive coronavirus variant was associated with a more severe course of the disease and more frequent hospitalization of children, and the long-term complications of the infection are unknown.[53] It is also extremely important during the COVID-19 pandemic to administer mandatory and recommended vaccinations to children in order to protect them from other infectious diseases. Unfortunately, there are reports of a decrease in their administration as early as 2020.[53–55]

Health and social consequences of the pandemic for children

Significantly fewer children than adults were infected with SARS-CoV-2. However, many more children have experienced the effects of isolation, repeated school closures, and prolonged negative mental and emotional states. Just over 2 years after the onset of the COVID-19 pandemic, another silent pandemic is beginning to emerge – obesity, depression and visual impairments.[56]

Children, like adults, were accompanied by permanent fear and uncertainty about the future. These feelings were compounded by isolation and the transfer of all social contacts to the Internet. A sedentary lifestyle, caused by prolonged time in front of the computer during remote activities, and the temporary inability to leave the house, contributed to weight gain and the intensification of compulsive overeating in many individuals.[57] These phenomena were particularly exacerbated in women and obese individuals.[57] Social problems of teenagers increased slightly to moderately during the pandemic.[58] A lack of companionship on weekdays and low activity increased depression and anxiety, especially among older adolescents.[59] They experienced significantly higher levels of separation anxiety and a fear of physical injury compared to other types of anxiety.[60] Their manifestations were most often fits of crying (32.8%), anger (28.6%) and aggression (26.7%).[60] These symptoms became more frequent during the lockdown, when harmful oral health and hygiene habits (finger sucking, nail biting and lip biting) occurred at a lower frequency after the lockdown than before it.[60]

Educational inequalities related to differing access to technology (only 20.0% of rural households were connected to high-speed Internet in 2020) and students' different levels of motivation for self-education, ranging from 30.0% to 42.0%, also worsened the situation.[61] Eighty-nine percent of parents described the quality of distance learning as worse, including 62.0% who reported a much worse quality of distance learning than classroom education.[61] A long time spent in front of screens and low physical activity also contributed to visual impairments and a higher prevalence of myopia in children aged 5–10 years.[62] An increase in the incidence of pediatric trauma and self-harm has also been reported.[63=]

A reduction in access to dental appointments should also be mentioned, which offers a worrying prospect for the coming years.[64] Due to the high risk of SARS-CoV-2 infection in the dental office, caused by direct face-to-face contact with patients and the presence of aerosols, alternative methods for dental visits were necessary during the pandemic.[65] Scheduled dental appointments and procedures had to be postponed, and only emergency treatments were implemented.[65] One of the alternatives that began to be more widely used is teledentistry.[65] With this method, it is possible to reduce the negative effects of the pandemic on the oral health of children and adults by providing adequate services without any risk of virus transmission. In addition, teledentistry, as a method with proven high efficiency, can be used in the future as one of the components of the healthcare system, enabling proper diagnosis and classification of patients, and thus reducing queues and the time to implement treatment.[65]

Among the positive changes, children's eating habits changed favorably, with more frequent consumption of fruits and vegetables, and an effort to reduce sugar and fat intake.[66]

In sum, the changes have concerned practically every sphere of children's lives and have not brought an optimistic prognosis for the future. The most important effects of the COVID-19 pandemic on children are shown in Table 1.

Conclusions

The COVID-19 pandemic has had an enormous impact on children. Even if they were not infected with the virus, their lives were changed by the nationwide quarantine and a sudden alteration of daily habits. The SARS-CoV-2 virus turned out to be relatively sparing in this age group, allowing children to pass through the disease mildly, often asymptomatically, with much lower hospitalization and mortality rates than those in adults. The main symptoms of the infection were respiratory, including fever, cough and a runny nose. In addition, children showed gastrointestinal and neurological symptoms, as well as dental problems (ulcers, blisters, inflammation of the oral cavity). Numerous uncertainties, isolation and significant changes in the education system have negatively affected the mental and physical health of the pediatric population. Weight changes, reduced physical activity, and increased social and emotional problems are bound to have a negative impact on their future lives. The deterioration of eyesight and dentition in the pediatric population seems worrying. Through remote learning, inequalities in children's education have widened, and the quality of their educational process has declined.

It should be remembered that the pandemic is not over yet, and currently (March 2022), a reduction in the level of precautions and the lifting of all sanitary restrictions have been implemented. Additionally, it is worth noting that all research results refer to a fairly short, two-year period. Currently, only the short-term effects of the pandemic among children are known, and concerns about the future remain in the realm of conjecture. In addition, due to the mostly benign and non-specific course of SARS-CoV-2 infections, not all cases of sick children were reported to doctors. Thus, the exact incidence statistics are not known, and a full qualitative and quantitative analysis of symptoms is impossible. The abovementioned facts are the limitations of the current study. In order to thoroughly analyze the condition of patients and assess complications of the COVID-19, it will be necessary to conduct further observations and research over a period of several years. Most of the negative effects of the COVID-19 pandemic on the pediatric population will not be observable until several years from now, but the fight against already identified problems and the prevention of foreseeable complications should begin immediately.

Ethics approval and consent to participate

Not applicable.

Data availability

All the data analyzed during the review process is included in this published article.

Consent for publication

Not applicable.

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and Medical Problems