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Diagnostic Criteria for Temporomandibular Disorders (DC/TMD): Polish assessment instruments

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Keywords: DC/TMD, temporomandibular disorders, TMD, diagnostic criteria

The article presents the Polish version of the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD), the process of document translation and cultural adaptation.

Temporomandibular disorders (TMDs) constitute a heterogeneous group of conditions characterized by the presence of signs and symptoms associated with the masticatory system, such as pain in the masticatory muscles and/or the temporomandibular joints (TMJs), limited jaw movements, and TMJ sounds (i.e., clicking and/or crepitus) during function.[1] Temporomandibular disorders pose a significant public health problem, affecting approx. 5–12% of the population, based on the United States estimates,[2] and likely have the same prevalence in Poland. Our understanding of the etiology, diagnosis and treatment of TMDs continues to improve. An essential part of the methodology facilitating this development is the use of standardized classification systems for ascertaining the case status, and for further investigating the disorders in terms of mechanisms and taxonomic improvement.

The first evidence-based diagnostic method for TMDs emerged in 1992 as the Research Diagnostic Criteria for TMDs (RDC/TMD).[3] The RDC/TMD came from the openly acknowledged need for a diagnostic system that could not only reliably distinguish cases from controls for epidemiologic and clinical research purposes, but also differentially define and diagnose the common subtypes, such as pain-related TMDs and mechanical disturbances within TMJs. The RDC/TMD consisted of 2 axes: Axis I for a physical diagnosis; and Axis II for assessing the behavioral and functional status of the patient. The RDC/TMD utilize epidemiologic data to determine at a population level the threshold distinguishing a disorder from ordinary symptoms, such as a transient pain process or TMJ clicking that exhibits no symptoms or functional consequences. In the subsequent 2 decades, the RDC/TMD evoked a significant response to these foundational principles from the international scientific community – the research built on testable evidence in the context of an iterative process, providing a basis for reliable and valid revisions that were to come next.[4]

The RDC/TMD instrument utilizes both self-reported and clinical examination data. While the instrument was developed in English, it served as a source for more than 20 approved translations over the 10 years following its development, which included Polish among the languages. The RDC/TMD were replaced by the Diagnostic Criteria for TMDs (DC/TMD), which represent the current reference standard for a reliable and valid diagnosis of the prevalent TMDs for both clinical and research use.[5] While the DC/TMD are not, as of yet, a mandatory protocol in any national patient care guidelines of which we are aware, it is the de facto standard for the clinical examination and diagnosis of the prevalent non-odontogenic disorders affecting the masticatory system.[6,7]

A key feature of both the RDC and DC approaches to TMD assessment is their dual-axis structure. Axis I, which concerns the physical domain, includes guidelines for oral history taking, as well as the clinical assessment of the joints and jaw muscles, and it leads to an algorithm-based diagnosis of the prevalent TMDs. Axis II, which concerns the psychosocial and behavioral dimensions, comprises instruments for the evaluation of pain intensity, pain-related disability, the functional limitation of the masticatory system, oral overuse behaviors, depression, anxiety, and the extent of multi-determined physical symptoms throughout the body. The content of the DC/TMD is presented in Table 1.

Table 1. Content of the English and Polish versions of the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD)

Axis	English	Polish
Axis I	TMD Pain Screener	TMD badanie przesiewowe bólu
	Symptom Questionnaire	Kwestionariusz objawów
	Demographics	Dane demograficzne
	Examination: Pain-related Interview and Examiner Commands	Badanie: wywiad dotyczący bólu i polecenia lekarza badającego
	Examination Form: International	Formularz badania klinicznego (wersja w języku angielskim)
	Decision Tree and Diagnostic Criteria Table	Drzewo decyzyjne i tabela kryteriów diagnostycznych (wersja w języku angielskim)
Axis II	Pain Drawing	Schemat bólu
	Graded Chronic Pain Scale (GCPS), version 2	Skala bólu przewlekłego (GCPS), wersja 2
	JFLS-8	JFLS-8
	JFLS-20	JFLS-20
	PHQ-4	PHQ-4
	PHQ-9	PHQ-9
	GAD-7	GAD-7
	PHQ-15	PHQ-15
	Oral Behaviors Checklist	Lista kontrolna parafunkcji

JFLS-8 – Jaw Functional Limitation Scale-8; JFLS-20 – Jaw Functional Limitation Scale-20; PHQ-4 – Patient Health Questionnaire-4; PHQ-9 – Patient Health Questionnaire-9; GAD-7 – General Anxiety Disorder-7; PHQ-15 – Patient Health Questionnaire-15.

Both the English and the Polish version of the DC/TMD are available on the INfORM (International Network for Orofacial Pain and Related Disorders Methodology) website: https://ubwp.buffalo.edu/rdc-tmdinternational/tmd-assessmentdiagnosis/dc-tmd. The examination procedures are available as a detailed document with explanations and illustrations,[8] and the examination process is fully presented in a video.[9] The Polish translation package includes Axis I and Axis II instruments and diagnostic algorithms (as listed in Table 1), complete specifications for a clinical examination, and a scoring manual for self-reporting instruments.

The English source version of the DC/TMD was formally translated into Polish – the target language – following the guidelines described in several documents.[10–12] They embrace guidelines for establishing the cultural equivalency of instruments, which describe how to create a valid translation of an instrument designed to collect research-quality data,[10] guidelines on the translation and adaptation of the DC-TMD protocol, which highlight specific translation challenges with regard to the DC/TMD,[11] and guidelines on the translation and review process (step by step, with explanatory notes), which further illustrate the procedures involved in the rigorous translation.[12] The development of a valid translation consists of 10 stages, with each stage resulting in written documentation via the translation log:

1. forward translation by 2 independent translators whose first language is Polish;
2. independent resolution of discrepancies between the 2 forward translations and their synthesis;
3. backward translation by 1 independent translator whose first language is English;
4. independent review of the backward translation vs. the source document by a medical translation professional;
5. revision and iterative development related to discrepancies that require repeating the forward and backward translations of the indicated parts, followed by an independent review;
6. after approval from an independent reviewer, consolidation of all translation and review activity into a single instrument appropriate for an internal review;
7. assembly of an expert committee comprised of 4 individuals and review of the translation quality by each committee member;
8. construction of a pre-final instrument;
9. independent review of the translation process and documentation; and
10. posting the translation on the INfORM website so that others can begin to contribute to Phase II: Translation Validation and Documentation.[10]

Specific details further highlight the rigorous process required for creating a medical instrument appropriate for both patient care and research. For the Polish translation, there were 2 forward translators whose first

language is Polish – one was informed or aware of the health concepts intrinsic to the DC/TMD, and the other was uninformed or naive of those concepts. This ideal allocation of the knowledge regarding the instrument ensures that the final translation adheres to both technical accuracy and the common-sense usage of Polish. Each forward translator produced an independent translation from the source language into the target language. The team leader reviewed both forward translations and resolved any differences in the translation style to facilitate the synthesis of the 2 translation versions. The backward translation of the synthesized forward translation was done by 1 independent translator whose first language is English and whose second language is Polish. The backward translator was totally blinded to the original source. The source and the backward translation were submitted for simultaneous evaluation by an independent reviewer, a medical translation professional who works across multiple areas of medicine and whose first language is English – the language of the source instrument. This particular reviewer was also highly experienced with the content and purpose of the source instrument. The reviewer identified the potential translation problems with regard to the backward translation, adding comments on the nature of the discrepancies. This review was sent back to the team leader. During revision and iterative development, the team leader coordinated the repeated forward and backward translations of the problematic areas until the independent reviewer approved the backward translation as the evidence of an acceptable forward translation.

The team leader then created a consolidated version of the approved forward-translation parts for each instrument, and the necessary comments were added for each item. An expert committee for cultural equivalency review was created; the committee consisted of 4 members, not involved in any of the prior steps: 3 of them were clinicians, and one was a language expert. Each committee member independently reviewed the forward translation against the source and the backward translation for the suitability of content within the context of the Polish culture. Recommendations were made with respect to 4 areas: semantic, idiomatic, experiential, and conceptual equivalence. The expert committee members suggested, as needed, further changes to the translation to ensure cultural appropriateness. Collectively, the review of an expert committee leads to cultural validity. The recommendations were submitted to the team leader, who incorporated the concerns and suggestions into the final forward translation.

On the home stretch, the team leader created the final draft of the instrument and compiled the translation logs. The final instrument draft serves for initial administration and the subsequent empirical testing. All documentation was sent to the Chair of Committee on Translation at INfORM, who reviewed the materials and determined whether the documentation for the translation process was acceptable. After the INfORM review was completed and the translation was approved, the Polish DC/TMD were posted on the INfORM website (https://buffalo.app.box.com/s/4ujx1lvndnxtw4suzvk2ymu9xhe8a5oz).

Although the accumulation of evidence over the years has been a strong argument against invasive and irreversible therapeutic TMD procedures, the biopsychosocial model of TMDs is still not fully accepted by all clinicians in Poland. It is partly due to the lack of knowledge on how to effectively implement the model.[13] Hence, this article aimed to introduce the operationalized tools for both a reliable and valid clinical examination leading to the diagnosis and psychosocial assessment of patients with TMDs. The DC/TMD provide the structure for the "bio" with Axis I diagnoses for physical disorders, and the structure for the "psycho" and "social" with Axis II tools for the assessment of the psychosocial profile. This approach is recommended for use in all patients with a potential TMD diagnosis.

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Real-world effectiveness of fremanezumab in patients with migraine switching from another mAb targeting the CGRP pathway

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This is a summary of the research article entitled "Real-world effectiveness of fremanezumab in patients with migraine switching from another mAb targeting the CGRP pathway: A subgroup analysis of the Finesse Study".

The discovery of calcitonin gene-related peptide (CGRP) as a therapeutic target in migraine has been one of the greatest achievements in neurology in recent years. Specific antibodies against CGRP bind to it either via a receptor (erenumab) or ligand (fremanezumab, galcanezumab, eptinezumab). Monoclonal antibodies (mAbs) are effective, safe and well-tolerated drugs that have been approved for prophylactic treatment if there are at least 4 days with migraine per month. However, in clinical practice, the failure of treatment with mAbs has been observed, and thus the question arises whether it is worthwhile to include treatment using an antibody with a different mechanism of action.

The Finesse Study was designed to evaluate the efficacy of fremanezumab in patients with a history of prior treatment failure with other mAbs against the CGRP pathway. Among the 153 patients with priorly failed mAbs, switching to fremanezumab led to a ≥50% reduction in the number of days with migraine per month in 42.8% of patients. The conclusion emphasizes that switching to another antibody should be considered in patients with prior therapy failure.

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Introduction

This article summarizes an observational, prospective, two-country study of fremanezumab treatment outcomes entitled "Real-world effectiveness of fremanezumab in patients with migraine switching from another mAb targeting the CGRP pathway: A subgroup analysis of the Finesse Study".[1] That research paper evaluates the efficacy of fremanezumab – one of 4 anti-calcitonin gene-related peptide (CGRP) monoclonal antibodies (mAbs) – in migraine patients with prior anti-CGRP pathway mAb treatment.

Study design and results

The study recruited 1,071 patients, but eventually included 867, as the remaining patients did not have complete data. All patients were treated with fremanezumab monthly (225 mg) or quarterly (675 mg).

Of these, 153 patients (episodic migraine (EM) – 52.3%, chronic migraine (CM) – 47.7%) had been previously treated with erenumab and/or galcanezumab:
– erenumab 70 mg (60.8%);
– erenumab 140 mg (71.9%);
– any erenumab (94.8%);
– galcanezumab (10.5%).

Primary endpoints – proportion of patients with a ≥50% reduction in monthly migraine days (MMDs).

Secondary endpoints – effectiveness of fremanezumab in terms of:
– changes in MMDs;
– impact on disease-induced disability (Migraine Disability Assessment (MIDAS), Headache Impact Test (HIT-6));
– use of acute medications.

Results after 3 months of treatment with fremanezumab

1. Reduction in MMDs and responder rates:
– a ≥50% reduction in MMDs → 42.8% of patients (a response rate of 48.0% in EM patients and 36.5% in CM patients);
– a ≥30% reduction in MMDs → 58.7%;
– MMDs decreased from 13.6 ±6.5 to 7.2 ±5.5 (a greater reduction in CM patients).

2. Migraine disability:
– the MIDAS scores decreased from 73.3 ±56.8 to 50.3 ±52.9;
– the HIT-6 scores decreased from 65.9 ±5.0 to 60.9 ±7.2.

3. Acute medication use:
– in all patients, it decreased from 9.7 ±5.0 to 4.9 ±3.7 days per month;
– in EM patients, it decreased to 3.8 ±3.1 days;
– in CM patients, it decreased to 6.3 ±3.9 days.

What was the discussion of the key results of the study?

– Erenumab was approved by the European Medicines Agency (EMA) earlier than ligand-acting mAbs; therefore, most of the included patients had previously undergone therapy with this mAb.
– Studies evaluating the switching of antibodies from different groups are few, and limited to single cases or retrospective studies.
– Differences in the efficacy of mAbs are attributed to their mechanisms of action, including effects on the blood–brain barrier (BBB).
– Functional magnetic resonance imaging (MRI) studies showed different responses of the central nervous system (CNS) to the ligand and receptor antibodies. Galcanezumab reduced activity in the left thalamus, hypothalamus and bridge areas, while erenumab specifically reduced activation in the insula, thalamus, cerebellum, and operculum.
– It seems that a large number of patients and broad inclusion criteria for patients with comorbidities better reflect real situations than phase 3 clinical trials.

What are the key practice points for clinicians?

– Different mechanisms of action of mAbs may affect their efficacy, safety and/or tolerability in patients with migraine.
– Patients who have not responded to one class of mAbs may benefit from switching to another class.
– It seems that a switch to mAb with a different mechanism of action would be most beneficial.

What are the perspectives for further research?

– The determinants of a response to a particular class of antibodies are still under investigation. There are more and more real-life studies suggesting that certain personal characteristics, migraine features, and comorbidities determine better responses to treatment, but long-term observations based on large groups of patients are needed.[2–4]
– An important issue in the coming years will be the development of guidelines for the duration of prophylactic treatment, including the determination of the time point after which anti-CGRP drugs can be considered ineffective.[5,6]

– Future studies should also answer the question of whether combining prophylactic therapies in a single patient can improve treatment efficacy and which combinations would be most beneficial.[7]
– Research is currently underway to identify new targets for migraine treatment.[8,9]

Prior presentation

This is a summary of a peer-reviewed article published previously in the Journal of Headache and Pain (https://doi.org/10.1186/s10194-023-01593-2).

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Original paper

Impact of working and learning from home during the COVID-19 pandemic on the head, the neck and orofacial health: A cross-sectional pilot study

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Abstract

Background. During the coronavirus disease 2019 (COVID-19) pandemic, about 81% of the world's population moved their workplace to a home office.

Objectives. The main objective of this cross-sectional pilot study was to determine the impact of working and/or learning from home during the COVID-19 pandemic on the head, the neck and orofacial health in university students, faculty and staff.

Material and methods. Participants from 4 universities were recruited for an online survey. The survey included 33 questions related to demographics, health issues before and during the lockdown, work/study from home, and the awareness of the health effects of the lockdown. Descriptive statistics and single logistic regression analysis were employed.

Results. A total of 96 subjects aged 26 ±10.5 years participated in the study. Of these, 60% did not consider their home workstation to be adequate. The development of new symptoms or the worsening of the pre-existing symptoms was observed in 67%, 24%, 59%, and 37% of the participants with regard to neck pain, temporomandibular joint (TMJ)-related issues, headaches, and parafunctional oral habits, respectively. In addition, 87% of the respondents reported that their oral habits were aggravated by neck pain and a bad posture. As compared to the faculty and the staff, the students were more likely to experience headaches or the exacerbation of the pre-existing headaches during the pandemic. In the survey, 91% of the participants reported an increased awareness of the impact of the lockdown on their head and neck, and orofacial health.

Conclusions. The present study helps understand the self-perceived effects of working and/or learning from home during the COVID-19 pandemic, and may facilitate implementing the appropriate models of treatment of the craniocervical-mandibular region during a pandemic.

Keywords: orofacial pain, COVID-19 pandemic, pandemic lockdown, head and neck posture

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Introduction

In the middle of March 2020, the world declared a mandatory lockdown due to the coronavirus disease 2019 (COVID-19) pandemic. Consequently, universities implemented remote learning and teaching methods. University students, faculty and staff had to study/work from home. It was estimated that 81% of the world's population transitioned from onsite to remote work in a home office during the lockdown.[1] Although work from home ensured comfort and safety, it also resulted in many adverse effects on physical and mental health.[2] A decrease in physical activity, increased sitting time and a decline in mental well-being are evidenced in self-report studies conducted among university students in the United States, the United Kingdom, Australia, Italy, and Spain.[3–7]

The COVID-19 pandemic was found to cause an increase in orofacial pain, including the intensification of bruxism (tooth grinding and/or clenching) and temporomandibular disorders (TMD).[8] In the long run, the lack of an ergonomic office set-up at home may promote a faulty posture, which makes an individual more susceptible to neck pain, TMD, and orofacial pain.[1] In a self-report study, 57% of participants declared using a four-leg kitchen chair without an adjustable height feature as part of their home office furniture.[8] About 86% of respondents reported using a single non-adjustable tabletop, and the majority reported using a chair with a concave back. Only 60% of participants used in their home office a monitor that had adjustable height.[8] Alterations in the head and neck posture can affect the forces exerted by the neck muscles, which must work harder to support the head in an unfavorable position.[9] The forward head posture (FHP) can alter the position of the mandible, which in turn displaces the temporomandibular joint (TMJ) condyles to a more posterior position, compressing the innervated area of the joints and causing pain.[10] In addition, the hyperactivity of the masticatory muscles has been observed for FHP when compared to a normal head posture.[10]

During the COVID-19 pandemic, an increased incidence of depression, anxiety and parafunctional habits was noted. Studies show a strong correlation between adverse psychological effects and orofacial pain, including TMD.[8,11] Patients with TMD were found to experience higher levels of stress, and report higher intensity of pain and pain-related disability.[12,13] An increase in myofascial pain in the masseter and temporalis muscles was observed in 78% of patients with TMD.[14] Stress, anxiety and depression exacerbated parafunctional habits and sleeping difficulties, which are associated with TMD.[14]

University students are particularly vulnerable to stress, anxiety and depression due to the lack of coping mechanisms. Most university students live away from home[15] and do not have a partner to help them manage their emotions, which may lead to higher levels of stress. University undergraduate students were affected differently by the COVID-19 pandemic than the working population, as the uncertain outlook added to their already stressful academic obligations.[15] Additionally, university employees reported increased levels of stress and anxiety, and many admitted they lacked the proper coping strategies.[16] The decline in their mental health was likely due to social isolation, managing their student's emotions and having to monitor their children' online learning at home.[16]

To the best of the authors' knowledge, no previous study has explored the impact of the COVID-19 pandemic on the head, the neck and orofacial health in university students, faculty and staff. The objective of the present study was to determine the impact of working and/or learning from home during the COVID-19 pandemic on the craniocervical-mandibular system, including the head and neck posture, and the associated orofacial pain, including TMD, in university students, faculty and staff. It was meant to help understand the self-perceived effects of working and/or learning from home during the COVID-19 pandemic. The study may be used by clinicians to facilitate implementing the appropriate models of treatment, including the use of telehealth,[17,18] and the prevention of health-related problems involving the craniocervical-mandibular system in the period of a pandemic lockdown.

Material and methods

Study design

This cross-sectional, descriptive pilot study was approved by the Institutional Research Board at Florida International University, Miami, USA (approval No. IRB-21-0247), and followed the recommendations of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Subjects and recruitment

A convenience sample of undergraduate and graduate students, faculty, and staff from 4 universities in the Miami-Dade County, FL, USA (Florida International University, University of Miami, Miami Dade College, and Barry University), participated in this study. The main aim of this survey was to generate hypotheses for future research; thus, a formal sample size calculation was not required. However, according to the literature on the sample size for survey studies,[19–21] a sample size of approx. 100 participants is required if a 10% sampling error is accepted. Also, Krejcie and Morgan suggested that a sample size needed to be representative of a given population.[22]

In this context, a theoretical target population of 2,000 would require 322 participants. Since this was an exploratory pilot study involving 28% of this target sample, the size included was considered acceptable.

Recruitment posters were distributed around the university campuses, and the participants could access the survey through a QR code. The participants were also reached through social media platforms (Reddit, Facebook and Instagram) and email. The link to the survey was provided in the message to the potential participants. The survey was open for data collection from September 2021 to March 2022. Prior to completing and submitting the survey, the participants provided informed consent to participate in the study and authorized the researchers to analyze their responses. The participants were assured that no identifiable information would be published or released, and that participation was voluntary. In addition, they were informed that they would not receive any compensation for participating in the study.

Questionnaire

A questionnaire was created using the Qualtrics online survey software (Qualtrics, Provo, USA). The survey included 33 questions related to demographics, health issues before and during the lockdown, work/study from home, and the awareness of the health effects of the lockdown (the survey is available on request from the corresponding author). A variety of multiple-choice, ranking-scale and open-ended questions were included in the survey. The frequently used 5 multiple-choice alternatives (always, very often, sometimes, rarely, and never) were selected to improve the sensitivity of data and decrease bias. The final questions were chosen through discussions among the research team and were based on previous research evaluating the effect of the pandemic on orofacial pain, including TMD, and mental health.[9,12–15] The main craniocervical-mandibular outcomes considered were neck pain, headaches, TMJ/jaw pain, and oral habits. The survey was reviewed and tested by the team by answering the questionnaire on Qualtrics to gather feedback for improvement and to refine the survey. Changes to the survey were implemented based on the received feedback. The questionnaire was considered to have face validity (based on the intuitive feeling that a measurement seems to be valid), and the assumption that the measurement is valid at face value was accepted.[23] The online survey was designed to be completed in no longer than 10 min.

The survey contained questions related to the perceived stress, anxiety and depression based on the Perceived Stress Scale.[24–26] These questions might help understand the potential relationship between the psychological effects of the COVID-19 pandemic and orofacial health, including the possible increase in orofacial pain and TMD.

Statistical analysis

Descriptive statistics were used to analyze the responses. Data was presented as the number of participants (n) and frequency (percentage). The open-ended question responses provided by some participants were also considered and presented under their respective topics. A cross-tabulation analysis was conducted using the Qualtrics analysis software. In addition, we explored univariate associations between some of the variables. The χ^2 tests or univariate single logistic regression were employed to establish relationships between the onset/worsening of symptoms of neck pain, jaw pain, headaches, stress/anxiety, and oral habits and age, gender, the respondent status (students vs. faculty/staff), and the number of hours in front of the computer. The strength of the evidence against the null hypothesis (p-value) was interpreted based on the criteria proposed by Sterne and Smith.[27] Increasing evidence against the null hypothesis was defined by decreasing p-values. All responses available for each of the questions were analyzed. The Stata® statistical software, v. 17, was used for all analyses.

Results

Demographics and characteristics of the participants

A total of 96 subjects (N = 96) participated in this pilot study. The survey was completed by 96 subjects, but not all questions were fully answered by all. The majority of the participants were female (75.3%) with an age range of 18–69 years and an average age of 26 ±10.5 years. Of he total number of participants, 83.3% (n = 80) were students, and 16.7% (n = 16) were faculty and staff. The subjects' demographics and characteristics and shown in Table 1.

Table 1. Demographics and characteristics of the participants (N = 96)

Characteristics		Values
Age [years] *M* ±*SD*, *IQR*		26 ±10.5 18–69
Gender *n* (%)	M	23 (24.7)
	F	70 (75.3)
Students *n* (%)		80 (83.3)
Faculty *n* (%)		4 (4.2)
Staff *n* (%)		12 (12.5)

M – mean; *SD* – standard deviation; *IQR* – interquartile range; M – male; F – female.
Three participants did not provide information as to their gender.

Health issues before the lockdown

Before the lockdown, the majority of the respondents (54%) reported spending an average of 3–6 h per day in front of the computer. A total of 62% (n = 55) reported that they rarely or never had neck pain before the pandemic. Eleven percent (n = 10) reported having neck pain always or very often, and 27% (n = 24) reported experiencing it sometimes.

Only 14% (n = 13) reported having headaches always or very often before the pandemic. In contrast, 44% (n = 39) of the participants rarely or never had headaches before the pandemic.

The majority of the respondents (89%) reported rarely or never experiencing problems related to TMJs or jaw muscles before the pandemic. Only 8% (n = 7) self-reported that they had been diagnosed with TMD. No further information regarding the diagnostic tool and the professional who had made the diagnosis was collected. Only 14% (n = 11) reported always or very often experiencing bruxism (the grinding and/or clenching of the teeth). Over half of the participants, (58%, n = 43) reported rarely or never having parafunctional oral habits before the pandemic.

A total of 92% (n = 81) of the participants reported no history of TMD diagnosis or lock jaw. In relation to bruxism, 37% (n = 27) of the participants reported never having clenched or ground their teeth, and 22% (n = 16) reported infrequent clenching or grinding before the lockdown.

Home office changes and health issues during the lockdown

Of the total number of participants, 87% (n = 77) reported transitioning from onsite to remote work in a home office in full time, while 8% (n = 7) reported transition in half time after the pandemic started. Among those who reported moving their workplace to a home office, 95% (n = 79) reported that they felt they spent more time on their computer and cell phone than before the lockdown. A total of 84% (n = 73) reported spending at least 6 h in front of the computer per day during the pandemic. A total of 34% reported spending 8–10 h on the computer and the cell phone per day, while 22% reported more than 10 h. Additionally, about 60% (n = 50) of the participants felt that their workstation at home was not adequate for maintaining a proper posture in front of the computer

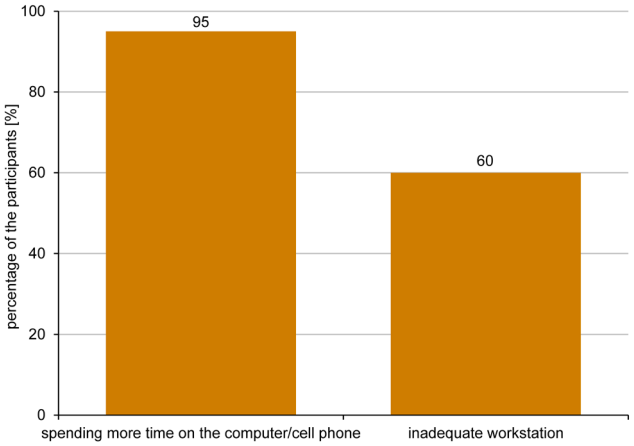

Fig. 1. Home office changes reported during the lockdown

(Fig. 1). When examining the correlation between the time spent in front of the computer and symptoms in the head, neck and orofacial regions, it was found that the individuals who spent more than 6 h in front of the computer had 4.68 times higher odds of developing neck pain or getting worse neck pain during the pandemic (p = 0.012). Similarly, the subjects who spent more than 6 h in front of the computer had 4.79 times higher odds of developing headaches or getting worse headaches during the pandemic (p = 0.015) (Table 2).

With the lockdown, 26% (n = 22) of the participants reported the onset of neck pain, and 41% (n = 35) reported the worsening of the already existing neck pain. Additionally, 37% of the participants reported the development or worsening of oral habits. The self-reported development of new symptoms and worsening of the pre-existing symptoms after the lockdown as compared to the state before the lockdown are shown in Fig. 2. The graph illustrates answers "always", "very often" and "sometimes" for before the lockdown data, and answers "yes, started during the pandemic" for after the lockdown (the onset of symptoms) data and "yes, my neck pain/headaches/oral habits/TMJ pain got worse during the pandemic" for the after the pandemic (the worsening of the already existing symptoms) data.

When examining the relationships between the onset/worsening of symptoms of neck pain, jaw pain, headaches, stress/anxiety, and oral habits and age, gender, the respondent status (students vs. faculty/staff), and the number of hours in front of the computer, weak evidence

Table 2. Statistical analysis results

Outcome	Variable of interest	*OD*	*CI*	*p*-value
Neck pain (onset/worsening of symptoms)	time spent in front of the computer (>6 h)	4.68	1.39–15.67	0.012*
Headache (onset/worsening of symptoms)	time spent in front of the computer (>6 h)	4.79	1.36–16.89	0.015*
Headache (onset/worsening of symptoms)	gender (F)	3.33	1.19–9.25	0.021*
Headache (onset/worsening of symptoms)	student	3.46	0.94–12.62	0.060
Stress/anxiety	gender (F)	3.08	1.06–8.85	0.037*

OD – odds ratio; *CI* – confidence interval; * statistically significant.

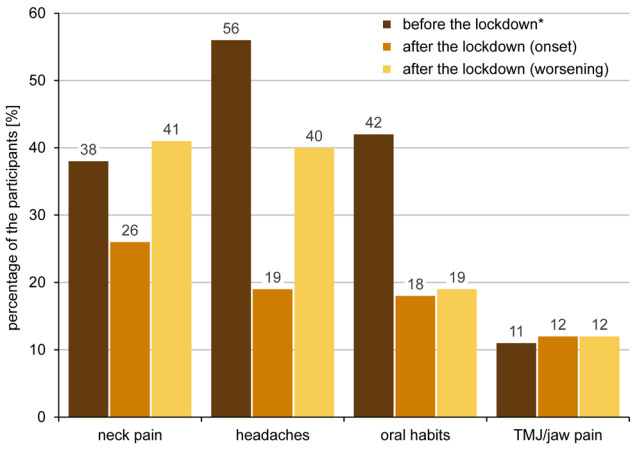

Fig. 2. Development of new symptoms and the worsening of the pre-existing symptoms after the lockdown

TMJ – temporomandibular joint; * answers "always", "very often" and "sometimes".

was found against the null hypothesis (of no association) in most cases. However, a significant association of the onset/worsening of headaches and stress/anxiety with gender was found. For example, during the pandemic, females were 3.33 times more likely to start with headaches or get worse headaches as compared to males (p = 0.021), and they were 3.08 times more likely to experience greater stress/anxiety (p = 0.037). There was moderate evidence against the null hypothesis, showing no significant association of the participant status (student vs. faculty/staff) with headaches. The students were 3.46 times more likely to start with headaches or get worse headaches during the pandemic than the faculty/staff (p = 0.060) (Table 2).

When asked whether their oral habits were aggravated due to neck pain or a slouched posture, 87% (n = 26) of the participants responded affirmatively. Additionally, when asked an open-ended question whether their TMJ/jaw pain was concomitant with neck pain, 5 out of 16 responded negatively. However, 9 were positive regarding the concomitant symptoms, with the answers including "Yes, I generally experience neck pain and jaw pain at the same time", "I think jaw pain is a direct result of tension in my neck and shoulders, which is due to the extended periods of a poor posture", "Yes, I think my posture affected my TMJ pain (I was always on the computer)", and "I usually get a headache, neck pain and jaw pain contractions when spending too much time in front of a computer". Two participants answered "I think TMJ/jaw pain was worsened by the stress related to my home situation" and "I believe it is because I started clenching my jaws and grinding my teeth more frequently".

Stress, anxiety, sleeping difficulties, and demotivation

Most of the responders (72%, n = 59) reported experiencing increased stress, nervousness and/or anxiety since the beginning of the lockdown (answers "yes, very often" and "yes, sometimes"). Only 19.5% (n = 16) reported feeling the same, and 8.5% (n = 7) reported feeling more relaxed during the pandemic. As previously stated, females were 3.08 times more likely to experience greater stress/anxiety during the pandemic than males (p = 0.037).

Of the total number of respondents, 53% (n = 43) reported they became unmotivated to work/study from home (answers "yes" and "very often"). With regard to demotivation, 25% said "sometimes," and 22% said "no". Thirty-nine percent (n = 33) reported developing sleeping difficulties with the lockdown (answers "yes" and "very often"), while 38% said "no," and 23% said "sometimes" in this regard.

When the participants were asked if they became fearful of their future regarding their studies/work, 42% (n = 34) reported "yes" or "very often," and 23% responded "sometimes". However, 68% (n = 56) were able to cope with the circumstances of the pandemic lockdown (answers "yes" or "most of the time"). Figure 3 shows the effects of the lockdown on stress/anxiety, sleeping difficulties, demotivation, and fear, as reported by the participants.

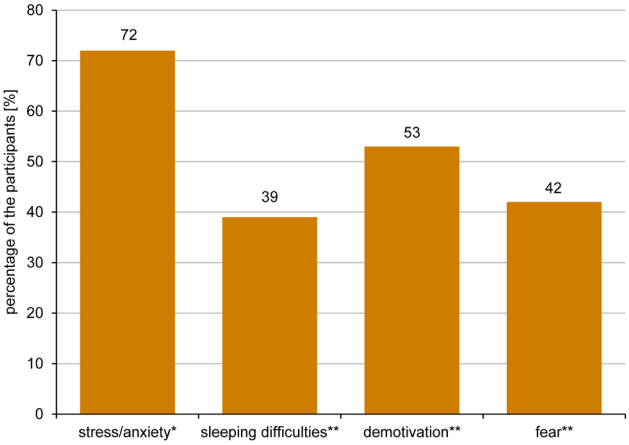

Fig. 3. Effects of the lockdown on stress/anxiety, sleeping difficulties, demotivation, and fear

* answers "yes, very often" and "yes, sometimes"; ** answers "yes" and "very often".

Self-awareness

The questions related to self-awareness were collected at the end of the survey. When asked if they were aware that a bad posture could potentially cause neck pain and possibly pain to the head, including the jaw area, 31% (n = 25) of the participants responded "No". Twenty-four percent (n = 19) of the respondents did not know that stress could cause parafunctional oral habits. After completing the survey, 63% of the participants agreed, and 28% somewhat agreed, that they were more aware of the possible impact of the COVID-19 pandemic on their general health, including the musculoskeletal conditions of the head and neck region (Fig. 4).

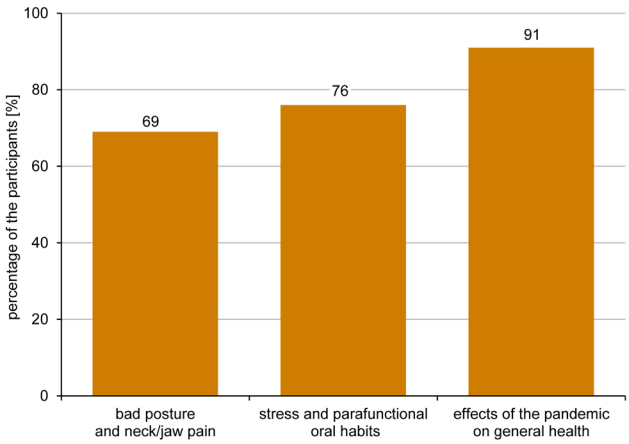

Fig. 4. Self-awareness regarding the relationships between a bad posture and neck/jaw pain, between stress and parafunctional oral habits, and the effects of the pandemic on general health (after completing the survey)

Discussion

This study investigated the self-perceived impact of working and/or learning from home during the COVID-19 pandemic on the head and neck region and orofacial health of university students, faculty and staff in the Miami-Dade area in Florida, USA. The study also aimed to increase the awareness regarding the relationships between a bad posture, stress, parafunctional oral habits, and pain in the head and neck region, which might be aggravated during the lockdown.

The pandemic lockdown gave rise to health threats, economic uncertainty and social isolation, causing potential damage to people's physical and mental health. According to the results of this study, 62% of the participants reported rarely or never experiencing neck pain, 44% rarely or never having headaches, 58% rarely or never having oral habits, and 89% rarely or never experiencing problems related to TMJ or jaw muscles before the pandemic. With the pandemic lockdown, the development of new symptoms or the worsening of the already existing symptoms, including neck pain, headaches, oral habits, and TMJ-related issues were substantial, as shown in Fig. 2. Additionally, females were more likely to develop or get worse headaches, and be more stressed during the pandemic than males.

Most participants regarded their home workstations as inadequate for maintaining a proper posture. They reported a more slouched head and neck posture as compared to the period before the pandemic, as well as spending more time on the computer and/or the cell phone. The subjects who spent more than 6 h in front of the computer were more likely to experience the onset or worsening of neck pain and headaches during the pandemic. Filho et al. found that the number of work breaks and hours spent at the computer had a great effect on neck pain, even though no clinically relevant changes in the average neck pain intensity and neck disability were found.[28] However, only 5 weeks of working from home was taken into consideration to investigate the effects on neck pain. The authors also found that there was very strong evidence that workstation ergonomics was poorer at home.[28] It is important to mention that the results can be referred to home offices that are not necessarily related to the COVID-19 pandemic. According to some open-ended question answers from our study, some participants reported using the kitchen or dinner table, and even the bed or couch while working/studying at home, constantly looking down at the laptop screen, and not having a comfortable chair or a chair that provided support to their lower back. One participant mentioned feeling a significant worsening of shoulder pain during the pandemic and holding more tension in their shoulders and neck. This may have contributed to increased pain around the craniocervical-mandibular region, including neck pain, headaches and TMJ-related issues. On the other hand, another study showed improvement in headaches during the COVID-19 lockdown.[29] According to the authors, the alleviation of migraines could be attributed to working from home and having fewer social responsibilities, as well as freedom to choose how to organize one's schedule.[29] Perhaps, the ability to adjust to changes while working from home also depends on an individual's lifestyle and general level of adaptability. In this survey, nearly 9% of the participants felt more relaxed during the pandemic.

In addition, oral habits and TMJ/jaw pain were found to be correlated to neck pain and a bad posture, as reported by most of the participants. The increased level of stress/anxiety during the lockdown, as reported by 72% of the participants, and increased sleeping difficulties (40%), may have also contributed to a higher frequency of parafunctional oral habits and, in turn, TMJ-related issues. Dentistry students declared dissatisfaction with distance learning during the pandemic, which may have contributed to their increased levels of distress.[30] In addition, in the present study, the students were more likely to start with or get worse headaches during the pandemic than the faculty and the staff. Previous studies have shown that the overwhelmingly quick transition from a normal lifestyle to a life of uncertainty during the pandemic lockdown could trigger increased levels of stress, anxiety and depression due to a fear of becoming infected with the virus, its impact on mental health and the concerns regarding personal finances.[11,12] Higher levels of psychological distress, anxiety and depressive symptoms were found in individuals with orofacial pain during the pandemic.[31] Even though not evaluated in this study, symptoms of anxiety and depression may affect the adaptability of patients with orofacial pain, which may lead to suicidal behavior.[32] Further studies are needed to examine the association between TMD/orofacial pain and suicidal behavior.[32] Some participants reported worsened sleeping patterns, and some weight gain. However, 19.5% of the participants felt the same levels of stress, nervousness and anxiety before and during the lockdown, and 8.5% reported feeling more relaxed during the lockdown. Physical activity appeared to play a role in helping people better cope

with the pandemic lockdown. According to open-ended question answers, some positive aspects of the lockdown reported by the participants included "Remote work has really made me deal with stress better", "Focusing on physical activity and health more", "The pandemic gave me years I needed to slow down and focus on improving my mental health. This has significantly improved my quality of life", and "The pandemic motivated me to exercise more, and it helped me to cope with the pandemic". The benefits of physical exercise are already acknowledged in the literature, and it is considered an effective approach to improving cardiorespiratory endurance in both people infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and non-infected individuals.[33] Physical exercise can also improve antibody responses and the effects of the influenza vaccine.[33] Identifying the activities performed by subjects to cope with the pandemic lockdown can be useful, and should be included in future studies.

Interestingly, 53% of the participants reported always or very often moving their jaw as a way to reposition the facial mask used during the pandemic. Of those reporting that their TMJ/jaw pain got worse during the pandemic, 71% admitted frequently adjusting the mask position in this way. Therefore, the use of masks may have increased the risk of worsening of the pre-existing TMJ/jaw-related problems. It is important to conduct additional studies on the effects of the use of masks on the repeated jaw movements and TMJ pain, as future pandemics are likely to happen. The increased awareness of the possible effects of mask use may help prevent mask-use-related issues.

In the present study, the majority of the participants agreed that the survey increased their overall awareness regarding the relationships between a bad posture, stress, oral habits, and pain in the head and neck region, which may be aggravated during the lockdown. This is important, as the risk of a new pandemic is higher now than ever before. Experts agree that the COVID-19 pandemic will likely not be the last one.[34,35] Therefore, we should learn from the lessons of the COVID-19 pandemic and make the necessary changes. The global health system should strengthen its capacity to prevent the effects of a lockdown and be prepared for the next public health emergency. This may involve putting more emphasis on physical activity, improving work ergonomics at home and increasing the awareness regarding the lockdown effects on health, including the head and neck posture and orofacial health. More investment in telehealth platforms is also recommended,[36] as it can help support the above recommendations based on the results of the present study.

Limitations

This study presents some limitations. The response rate was limited, and the majority of the responders were students. Faculty and staff were represented by only 17% of the total group, which limits the representation of this population in the sample and, in turn, the generalizability of the study. The results of this study should be interpreted with caution because of the small sample size and low external validity. To ensure maximum participation, the survey was designed to be brief, taking no more than 10 min to be completed. However, the authors assume that the time period during which the data was collected may have affected the recruitment of participants. Students, faculty and staff were gradually going back to their in-person routines, and most faculty and staff continued working remotely for the majority of their time. Therefore, not all of them had access to the recruitment posters around the university. The recruitment method may have affected the participation of several potential subjects, even though it was not the only method used. Other strategies to increase participation should be considered in future studies. The conducted analysis was limited by the small sample size. With a larger sample, further analysis (i.e., regression) could be performed. Therefore, this is a pilot study indicating the effects of working and/or learning from home during the COVID-19 pandemic on university students, faculty and staff. This study is based on self-reported data, and therefore, it can affect the properties of the measurements, including clinical validity and reliability, and it may be subject to recall bias, since the participants had to remember some past, though recent, events. The variables studied, including a bad posture, neck pain, oral habits, and psychological effects, were limited by the participant's knowledge and the clarity of the questions asked. This was a cross-sectional study, where data was collected at one point in time. However, the results of this study provide relevant information that can be used to guide more robust scientific research and the development of longitudinal studies, integrating further validated questionnaires, such as those for TMD or headache diagnosis, with larger sample sizes, and a better representation of faculty and staff. It can also be used to strengthen the prevention capacity with regard to the effects of a lockdown, based on the lessons learned from the COVID-19 pandemic.

Conclusions

This study helped understand the self-perceived effects of working and/or learning from home during the COVID-19 pandemic on the head and neck region and orofacial health. The findings revealed an increase in the onset or worsening of neck pain, headaches, TMJ/jaw pain, and oral habits in the university setting. The results may contribute to the development of the appropriate models of treatment of the craniocervical-mandibular region during pandemic lockdowns. The study also increased the awareness of the participants regarding the negative impact of the pandemic on physical and mental health, which may lead to decreased performance in work- or school-related activities.

Ethics approval and consent to participate

The study was approved by the Institutional Research Board at Florida International University, Miami, USA (approval No. IRB-21-0247). All participants provided written informed consent.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Evaluating and comparing the efficacy of the microsurgical approach and the conventional approach for the periodontal flap surgical procedure: A randomized controlled trial

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Abstract

Background. The use of the magnification approach for the periodontal flap surgical procedure helps in better visualization and better handling of soft tissues, which results in early wound healing.

Objectives. The aim of the present study was to compare the conventional macroscopic approach for periodontal flap surgery with the microsurgically modified approach in a randomized controlled clinical trial.

Material and methods. A total of 60 subjects were randomly divided into 2 groups: group A (test group), in which the subjects underwent the conventional open flap debridement procedure; and group B (control group), in which the subjects underwent open flap debridement with the use of a microsurgical loupe. The plaque index (PI), the gingival index (GI), the probing pocket depth (PPD), the clinical attachment level (CAL), and gingival recession (GR) were recorded at baseline, and at 3, 6 and 9 months postoperatively. Also, the early wound-healing index (EHI) was recorded at 10 days postoperatively.

Results. Both the conventional and the microsurgical technique provided a statistically significant reduction in PI, GI and PPD as well as gain in CAL. However, the microsurgical technique demonstrated a statistically significant decrease in postoperative GR as well as reduced pain perception and EHI scores.

Conclusions. The use of the microsurgical approach provides better clinical results with less discomfort, and thus makes the periodontal treatment more acceptable for the patient.

Keywords: chronic periodontitis, wound healing, microsurgery, periodontology

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Introduction

The treatment options for periodontitis depend on the severity of the disease and can be non-surgical, followed by a surgical intervention. To date, the non-surgical periodontal therapy has been considered as one of the most effective methods for the treatment of periodontitis.[1,2] The removal of the primary etiological factors, i.e., the microbial biofilm and plaque-retentive calculus, along with the removal of the altered cementum surface during the scaling and root planing (SRP) procedures leads to the reduction of pathogenic microbial load both at the tissue side and at the tooth side of the periodontal pocket.[3–5] However, the non-surgical periodontal therapy has its own limitations when it comes to deep periodontal pockets as well as deep intrabony defects. The outcomes of the treatment of moderate to severe periodontitis are better (a reduction in the probing pocket depth (PPD) and gain in the clinical attachment level (CAL)) when the cases are dealt with the flap surgical techniques along with the SRP therapy. Despite the meticulous efforts of the clinician to remove plaque and calculus in deep periodontal pockets, the complete eradication of local factors cannot be achieved. Periodontal flap surgery may facilitate access for the thorough decontamination of tissues, thus leading to better healing and better treatment outcomes.[6,7]

However, sometimes, the complete removal of local factors is doubtful even with the access flap (AF) surgical approach, especially when applied without using any magnification tools.[8,9] Waerhaug reported that at the depth of the periodontal pocket, deposits were found even after flap surgery.[10] It is difficult for the operator to localize plaque and calculus at the depth of the periodontal pocket due to the area being obscured by bleeding during the procedure.[10] Several in vitro studies have shown a range of 12–30% of residual calculus on the extracted teeth even after complete SRP.[11–13] Hence, it is reasonable to assume that the optical magnification of the surgical field might help in overcoming these issues.

The use of magnification in microsurgery leads to the refinement of the surgical techniques due to the enhanced visualization.[14] In microsurgery, viewing the finer details of the surgical field as well as using microsurgical instruments enable the operator to accurately and atraumatically manage the hard and soft periodontal tissues, which results in better debridement of the periodontal pocket, and thus a fully decontaminated surface, free of bacterial load, can be obtained.[15–18]

Based on these advantages, we tested the hypothesis that the improved visual acuity and better tissue debridement that result from the application of the microsurgical approach may improve the clinical outcomes of periodontal flap surgery, and thus provide less postoperative discomfort to the patient. Therefore, the objective of the present study was to compare the conventional macroscopic approach for periodontal flap surgery with the microsurgically modified approach in a randomized controlled clinical trial.

Material and methods

The present randomized controlled clinical trial comprised 64 patients who were affected by chronic periodontitis. The patients were selected at the Outpatient Department of Periodontology and Implantology in the Institute of Dental Sciences, Bareilly, India. The study protocol was reviewed and approved by the Institutional Ethics Committee (No. of approval: IDS.BIU/243/2019). After the evaluation of phase I (the non-surgical therapy), patients with a minimum of 20 natural teeth, exhibiting PPD ≥ 5 mm and CAL ≥ 5 mm were included in the study. Patients with known systemic diseases, allergies or drug use, pregnant or lactating mothers, and/or those who had undergone the periodontal therapy in the past 6 months were excluded from the study. After selection, the patients were informed about the purpose and duration of the study, and the written informed consent was obtained from them. As this was a parallel group study, the subjects were randomly divided into 2 groups, using the coin toss method. The group A (test group) subjects underwent open flap debridement with the use of a microsurgical loupe, whereas the group B (control group) subjects underwent the conventional open flap debridement procedure without any magnification tool. The patients were blinded to the type of local treatment they received.

The plaque index (PI),[19] the gingival index (GI),[20] PPD, CAL, and gingival recession (GR) were recorded at baseline (Fig. 1A,2A), and at 3, 6 and 9 months postoperatively with a UNC-15 graduated periodontal probe (Hu-Friedy, Chicago, USA). Additionally, the patient's pain perception was assessed with the use of the visual analog scale (VAS) and the healing of tissues was also evaluated by means of the early wound-healing index (EHI) at 10 days postoperatively.

Surgical procedure

Group A (test group)

At the test sites, a dental loupe of ×3.5 optical magnification was used to carry out the microsurgical flap procedure. After profound anesthesia was achieved with an anesthetic agent (2% lignocaine hydrochloride with adrenaline – 1:80,000), crevicular incisions were performed on the facial/buccal and lingual/palatal sides, reaching the tip of the interdental papilla with the use of microsurgical blade No. 15C (Hindustan Syringes & Medical Devices, New Delhi, India) (Fig. 1B). The buccal and lingual mucoperiosteal flaps were elevated with a micro periosteal elevator. Due to the enhanced visualization of the site, the unnecessary exposure of the bone tissue was avoided. Complete degranulation was done along with root planing with the use of a microsurgical curette (Hu-Friedy) (Fig. 1C). Flap approximation was achieved by using 5-0 monofilament polypropylene sutures (Centenial Surgical Suture, Murbad, Thane, India) (Fig. 1D).

Group B (control group)

Under proper aseptic conditions, the surgical site was anesthetized using 2% lignocaine hydrochloride with adrenaline (1:80,000). Using a Bard–Parker (BP) knife with blades No. 12 and 15 (Hindustan Syringes & Medical Devices), intracrevicular incisions were made on both the buccal and lingual/palatal sides (Fig. 2B). The full-thickness mucoperiosteal flaps were elevated, and the surgical debridement of the infected tissue and root planing were done, using appropriate Gracey curettes (No. 1–14) and 2R-2L, 4R-4L curettes (Hu-Friedy) (Fig. 2C). Flap approximation was done with 3-0 silk sutures (Centenial Surgical Suture) (Fig. 2D).

In both groups, a periodontal dressing was placed (Fig. 1E,2E), and the patients were prescribed a non-steroidal anti-inflammatory medication for 2 days and a systemic antibiotic for 5 days. The patients were instructed to rinse their mouths with 0.2% chlorhexidine gluconate twice daily for 2 weeks and to avoid any undue trauma to the treated site. The EHI was assessed 10 days postoperatively for both groups (Fig. 1F,2F).

Fig. 1. Surgical procedure for the test group

A – preoperative measurement of the probing pocket depth (PPD) (at baseline); B – incision marking; C – after flap reflection and debridement; D – 5-0 suture placement; E – periodontal dressing placement; F – early healing after 10 days; G – postoperative measurement of PPD (at 9 months).

Fig. 2. Surgical procedure for the control group

A – preoperative measurement of PPD (at baseline); B – incision marking; C – after flap reflection and debridement; D – 3-0 suture placement; E – periodontal dressing placement; F – early healing after 10 days; G – postoperative measurement of PPD (at 9 months).

Statistical analysis

The IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA) was used for the statistical analysis. The intragroup comparison was done using the Friedman test, with a p-value <0.05 considered as statistically significant. The post hoc analysis was performed using the Wilcoxon signed rank test and $p < 0.0008$ was considered as statistically significant (after applying the Bonferroni correction). The intergroup comparisons were done using the Mann–Whitney U test, with a p-value <0.05 considered as statistically significant.

Results

A total of 64 patients initially participated in this study, but 4 patients were lost to follow-up and excluded from the analysis. The remaining 60 patients suffering from chronic periodontitis were finally analyzed. The patients were divided into test group A (n = 30) and control group B (n = 30). Figure 3 summarizes the phases of the study.

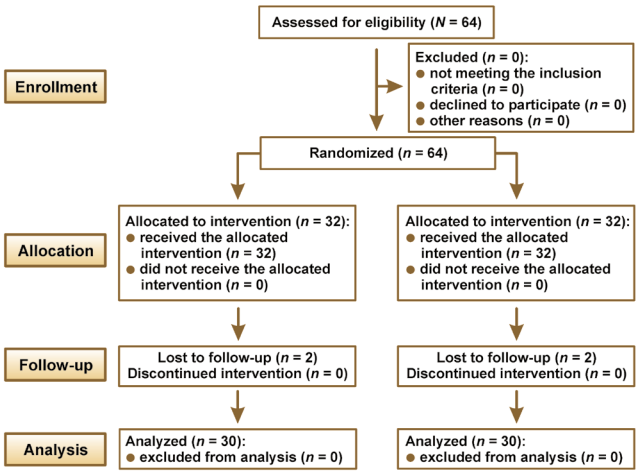

Fig. 3. Flow diagram of the randomized controlled clinical trial

In the present study, for both the test group and the control group there was a statistically significant decrease in the PI and GI scores from baseline to 9 months (Table 1). For the test group, a reduction in PPD was observed from the baseline value of 4.33 +0.36 mm to 2.81+0.40 mm at the recall visit at 9 months; for the control group, the values were 4.19 +0.40 mm and 2.99 +0.28 mm, respectively. The mean CAL value for the test group at baseline was 4.25 +0.36 mm and at 9 months, it was reduced to 2.75 +0.43 mm, whereas for the control group, it was 4.16 +0.39 mm at baseline and 3.00 +0.32 mm at 9 months. The gain in CAL and the reduction in PPD were statistically significant for both groups. When the position of the gingival margin was compared, the test group showed no statistically significant difference between the baseline and 9-month follow-up period values (p = 0.06), whereas the control group showed a statistically significant increase in the apical migration of gingiva during this time period (p = 0.00) (Table 1).

Table 2 shows the intergroup comparison between the test and control groups from baseline through every 3-month recall time period until 9 months. A greater reduction in PPD at 9 months was noted for the test group (2.81 +0.40 mm) as compared to the control group (2.99 +0.28 mm). When the CAL gain was considered, significant gain was recorded for the microsurgery group

Table 2. Intergroup comparison of the periodontal parameters at different time intervals

Parameters	Time periods	Test group	Control group	p-value
PI	baseline	1.10 +0.18	1.04 +0.24	0.23
	3 months	0.60 +0.21	0.54 +0.18	0.98
	6 months	0.79 +0.23	0.62 +0.18	0.01*
	9 months	0.90 +0.16	0.81 +0.21	0.11
GI	baseline	61.77 +21.71	63.44 +21.93	0.68
	3 months	23.60 +10.69	26.16 +13.32	0.41
	6 months	24.70 +11.38	28.41 +12.30	0.24
	9 months	30.06 +11.91	33.03 +13.88	0.35
PPD [mm]	baseline	4.33 +0.36	4.19 +0.40	0.08
	3 months	2.90 +0.35	2.97 +0.28	0.48
	6 months	2.80 +0.34	2.96 +0.29	0.04*
	9 months	2.81 +0.40	2.99 +0.28	0.05
CAL [mm]	baseline	4.25 +0.36	4.16 +0.39	0.24
	3 months	2.85 +0.36	2.94+0.28	0.31
	6 months	2.74 +0.35	2.94 +0.29	0.02*
	9 months	2.75 +0.43	3.00 +0.32	0.02*
GR [mm]	baseline	0.00 +0.00	0.00 +0.00	1.00
	3 months	0.00 +0.00	0.03 +0.18	0.31
	6 months	0.03 +0.18	0.43 +0.62	0.00*
	9 months	0.10 +0.30	0.80 +0.76	0.00*

* statistically significant (Mann–Whitney U test).

Table 1. Intragroup comparison of the periodontal parameters at different time intervals

Groups	Parameters	Baseline (n = 30)	3 months (n = 30)	6 months (n = 30)	9 months (n = 30)	p-value	Mean change [%]
Test group	PI	1.10 +0.18	0.60 +0.21	0.79 +0.23	0.90 +0.16	0.00*	18.18
	GI	61.77 +21.71	23.60 +10.69	24.70 +11.38	30.06 +11.91	0.00*	51.34
	PPD [mm]	4.33 +0.36	2.90 +0.35	2.80 +0.34	2.81 +0.40	0.00*	35.10
	CAL [mm]	4.25 +0.36	2.85 +0.36	2.74 +0.35	2.75 +0.43	0.00*	35.29
	GR [mm]	0.00 +0.00	0.00 +0.00	0.03 +0.18	0.10 +0.30	0.06	100.00
Control group	PI	1.04 +0.24	0.54 +0.18	0.62 +0.18	0.81 +0.21	0.00*	22.12
	GI	63.44 +21.93	26.16 +13.32	28.41 +12.30	33.03 +13.88	0.00*	47.94
	PPD [mm]	4.19 +0.40	2.97 +0.28	2.96 +0.29	2.99 +0.28	0.00*	28.64
	CAL [mm]	4.16 +0.39	2.94 +0.28	2.94 +0.29	3.00 +0.32	0.00*	27.88
	GR [mm]	0.00 +0.00	0.03 +0.18	0.43 +0.62	0.80+0.76	0.00*	100.00

PI – plaque index; GI – gingival index; PPD – probing pocket depth; CAL – clinical attachment level; GR – gingival recession; * statistically significant (Friedman test).

(2.75 +0.43 mm) as compared to the conventional flap surgery group (3.00 +0.32 mm) at 9 months. For the conventional flap surgery group, a statistically significant increase in the apical migration of gingiva was observed at both the 6-month (0.43 +0.62 mm) and 9-month (0.80 +0.76 mm) recall intervals as compared to the test group.

Table 3 shows the assessment of the patients' pain perception with the use of the VAS scores. A statistically significant reduction in the VAS scores was found for the test group (1.56 +0.77) as compared to the control group (4.23 +0.77), showing less postoperative pain for the microsurgical group. When healing was compared between the groups with EHI, significantly better results were found for the test group (1.53 +0.57) as compared to the control group (3.16 +0.46.) (Table 4).

Table 3. Intergroup comparison of the visual analog scale (VAS) scores

Parameter	Test group (n = 30)	Control group (n = 30)	p-value
VAS	1.56 +0.77	4.23 +0.77	0.00*

* statistically significant (Mann–Whitney *U* test).

Table 4. Intergroup comparison of the early wound-healing index (EHI) scores

Parameter	Test group (n = 30)	Control group (n = 30)	p-value
EHI	1.53 +0.57	3.16 +0.46	0.00*

* statistically significant (Mann–Whitney *U* test).

Discussion

This study compared the conventional macroscopic approach for the AF surgery technique with the microsurgically modified approach in a randomized controlled clinical trial and considered the hypothesis that the improved visual acuity and better soft tissue handling through the application of the microsurgical approach might improve the predictability of the clinical outcomes and provide less postoperative discomfort to the patients suffering from chronic periodontitis. The only variable of the study was the use of surgical loupes, microsurgical instruments and microsurgical suture material.

Irrespective of the procedure used for open flap debridement, there was significant improvement in the PI and GI scores from baseline to 9 months.

Similar to this, a statistically significant reduction in PPD was observed in both groups at 9 months (Fig. 1G,2G), though the improvement in the test group was slightly greater than in the case of the control group. As stated in previous studies, even after the non-surgical periodontal therapy or open flap debridement, residual calculus can be noticed on the root surfaces.[21] Microsurgery can lead to better clinical outcomes. Better visualization of the area increases the mechanical efficiency of SRP, and thus helps to eliminate the microbial etiological factors of periodontal disease, which results in better healing. Several studies have demonstrated the improved treatment outcomes with the use of the microsurgical technique when compared with the macrosurgically performed flap surgery.[18,22] In the present study, statistically significant gain in CAL was observed in the test group as compared to the control group. Although unwanted, gingival recession can occur post-surgically after the conventional flap surgical procedures.[23] In their studies, Becker et al. and Kaldahl et al. reported statistically significant post-surgical recession after applying several surgical treatment modalities, such as flap debridement, osseous surgery and root planing.[24,25] The periodontal pocket depth has been found to be directly correlated to gingival recession, i.e., deeper pockets may show more gingival shrinkage and more recession.[24] Various explanations for postoperative recession have been suggested, including the lack of bone support for the flap, thin gingival tissue with limited blood supply and the postoperative shrinkage of the flap. Esthetically unacceptable to the patient, this can also lead to dentinal hypersensitivity, and thus it should be controlled or kept at a minimal level.[24,26]

The present study shows significantly less gingival recession postoperatively in the cases treated with the microsurgical approach as compared to the conventional flap surgery approach. It is reasonable to assume here that the microsurgical approach may be related to a more predictable outcome because of the magnified vision of the surgical field. The unnecessary cutting and removal of tissues during surgical debridement is avoided, and hence trauma to tissues is reduced. This might lead to lesser gingival shrinkage. The vascular supply of the flap is improved, and there is a greater possibility of better flap adaptation, and also of better wound stabilization and healing.[27]

Using the microsurgical approach, better pain perception scores as well as better EHI scores were obtained for the test group as compared to the conventional flap surgery group. It might be due to the use of microsurgical instruments and micro-sutures.[28] Today, the patients envisage and expect an ideal form of periodontal therapy that is not only limited to the elimination of the bacterial component of the disease, but also improves the patient's appearance as well as function. The comfort of the patient increases if such treatment is provided with minimal trauma. The use of microsurgery does not change the periodontal therapy, but it increases the clinician's ability to accurately and gently handle tissues as well as the patient's acceptance of the treatment.

Every dental professional is at a potential risk for developing an occupational musculoskeletal injury. Magnifying loupes do play a major role in visualizing the minute details of the area without straining the eye muscles. The reported primary benefits of loupes refer to ergonomics and the proper posture, better evaluation/detection of the area, and thus overall, better treatment quality.[29] There are some disadvantages that limit the use of loupes by dentists, i.e., the lack of fixed position (the fine movements of the dentist's head disturb the image of the magnified surgical field) and the need to change loupes to achieve different magnification.

To overcome this, the surgical microscope guarantees a more ergonomic working posture, the optimal lighting of the operation area and freely selectable magnification levels. Since microscopes are external to the body, clinicians who use them are not affected by the weight of the instrument or the challenge of maintaining the stabilized field of vision. However, these advantages are countered by an increased cost of the equipment, and the extended learning phase for the surgeon and their assistant.

Conclusions

Both the conventional and the microsurgical techniques provided a statistically significant reduction in PPD as well as gain in CAL. However, the microsurgical technique demonstrated also a statistically significant decrease in postoperative GR as well as reduced pain perception and EHI scores. The variable of the study was the use of surgical loupes, microsurgical instruments and microsurgical suture material. Thus, these better results can be attributed to the use of the microsurgical approach for open flap debridement.

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Institutional Ethics Committee at the Institute of Dental Sciences, Bareilly, India (No. of approval: IDS.BIU/243/2019). The patients were informed about the purpose and duration of the study, and the written informed consent was obtained from them.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Evaluation of the effect of palatoplasty on the quality of life and speech outcomes in patients with velocardiofacial syndrome

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Conflict of interest
None declared

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Abstract

Background. Children diagnosed with velocardiofacial syndrome (VCFS) suffer from various disabilities. Palatal abnormalities, as well as speech and language impairment, adversely affect a child's quality of life (QoL) and are some of the most distressing aspects for the parents of these children.

Objectives. The present study aimed to explore the effect of palatoplasty on the health-related quality of life (HRQoL) and speech outcomes in children with VCFS.

Material and methods. The study recruited 20 patients ($N = 20$) with VCFS and connected speech, aged 3 years or older, having either undiagnosed submucous cleft palate (SMCP) or velopharyngeal insufficiency (VPI), and requiring primary cleft palate surgery or revision surgery. Speech assessment was conducted prior to palatoplasty and 6 months after the surgery. Intelligibility and hypernasality were evaluated using the Cleft Audit Protocol for Speech – Augmented (CAPS-A). The parent proxy-report form of the Pediatric Quality of Life Inventory (PedsQL™) was used to evaluate and compare the HRQoL of the VCFS patients before and after palatoplasty.

Results. Significant improvement in the HRQoL scores was achieved after the surgery across all domains (physical, emotional, social, and school functioning), especially in the emotional and social dimensions ($p < 0.000$). The post-operative speech assessment based on CAPS-A demonstrated improvement in speech intelligibility and hypernasality in the majority of patients.

Conclusions. Given that children with VCFS face various medical and social problems, suitable palatal interventions are beneficial, improving both the speech ability and QoL of these children.

Keywords: quality of life, DiGeorge syndrome, cleft palate

Introduction

Velocardiofacial syndrome (VCFS) is one of the most common examples of an autosomal dominant disorder caused by a micro-deletion of chromosome 22q11.2, affecting nearly 1 in 3,000 children.[1] The syndrome is diagnosed with an accurate and reliable special blood test called fluorescent in situ hybridization (FISH).[2] Children with VCFS display many problems that can be challenging to their parents and family members. Typical facial characteristics of the syndrome are posteriorly rotated ears or simple helices as external ear phenotypes. The permanent hearing loss associated with palatal anomalies has also been reported.[3] Of the 180 possible physical symptoms, palatal abnormalities are present in 69–100% of the affected children, with 9–11% having cleft palate, 5–16% having submucous cleft palate (SMCP) and 27–92% suffering from velopharyngeal insufficiency (VPI). Palatal anomalies, as the most prevalent features, result in a decreased physical function affecting the feeding abilities (i.e., growth retardation), a poor functional status and speech difficulties.[4] Effective communication and social interactions are directly associated with an individual's social well-being. Therefore, speech and language problems may adversely affect the quality of life (QoL) in children with VCFS, posing a major concern to their parents.[5,6] A previous study reported that a large proportion of people with a voice disorder experienced a decrease in QoL.[7]

Patients with chromosome 22q11.2 deletion syndrome (22qDS) commonly present with a large central velopharyngeal gap in the setting of poor velar and pharyngeal wall motion. The presence of a velopharyngeal gap can be identified based on a listener's judgment, oral examinations and sound production tests.[8] The Cleft Audit Protocol for Speech – Augmented (CAPS-A) is a validated and reliable tool to evaluate the speech of patients with cleft palate and VPI.[9,10] Due to advances in medicine, pediatric healthcare expands beyond the physical health of a child to include their health-related quality of life (HRQoL).[11,12] Researchers agree that QoL is a multi-dimensional concept that encompasses physical, psychological, social, and spiritual aspects.[13] The health-related quality of life can help translate a patient's experience of illness into quantifiable outcomes. Clinicians and researchers can determine the effectiveness of interventions to improve QoL in individuals with acute or chronic health problems. The quality of life in children with chronic conditions, such as VCFS, is affected by complex and dynamic interactions between various factors. Interactions between physical, emotional, social, and school functioning significantly influence both the overall well-being and QoL of a child.[14,15] The Pediatric Quality of Life Inventory (PedsQL™) offers a modular approach to measure HRQoL in healthy children and adolescents, and in those with acute or chronic health conditions.[16,17]

Since VCFS patients suffer from a wide range of problems, their speech impairment is often ignored in the light of other possible disabilities. Surgical protocols are contradictory, which complicates the treatment of VCFS patients. However, appropriate palatal surgery can be effective in enhancing patients' QoL. Hence, the present study aimed to explore the post-operative effect of palatoplasty on HRQoL and speech outcomes in children with VCFS.

Subjects and methods

The present study was conducted over 5 years (2014–2019) at the Cleft Lip and Palate Center of Shiraz University of Medical Sciences, Iran. The target population included patients with VCFS and those suspected of having the syndrome who were referred to the center. The FISH test was performed to confirm the diagnosis of VCFS in the suspected patients. The inclusion criteria were children with VCFS and connected speech, aged 3 years or older, having either undiagnosed SMCP or VPI, and requiring primary or revision repair. Of the initial 45 eligible candidates, 20 patients met the inclusion criteria and were recruited in the study. Prior to speech assessment, the parents of the children were informed about the research goals, the test procedure, and what was expected of them during the assessment. Written informed consent was obtained from the parents of the patients, and the anonymity of the children was guaranteed. The study protocol was approved by the institutional Ethics Committee at Shiraz University of Medical Sciences (IR.SUMS.REC.1398.535).

Speech assessment was carried out in 2 stages – prior to palatoplasty and 6 months after the procedure. In line with a previous study,[18] a digital voice recorder (DM-3; Olympus, Tokyo, Japan) and a camera (DCR-SR62E; Sony, Tokyo, Japan) were used to record the voice and image of the participants. The voice recorder was positioned at a distance of 15 cm from the patient's mouth, and the recordings were made in a quiet and well-prepared environment. Since the recordings before and after palatoplasty were taken by a third party, blind data analysis was ensured. The same person randomly organized, coded and stored the recordings on a digital optical disc. Subsequently, an independent speech–language pathologist, specialized in orofacial cleft malformation, speech intelligibility and hypernasality in such patients, analyzed the data from the 20 participants with the use of CAPS-A. To ensure accuracy, the data from 10 participants was randomly selected from the same dataset, re-analyzed and compared with the initial analysis.

In the present study, we evaluated the intelligibility and hypernasality parameters of CAPS-A. The assessment involves data interpretation based on colors; each color represents a level of speech difficulty: green – normal; yellow – needs further investigation and improvement; and red – unsatisfactory.[10] Intelligibility is the degree to

which speech can be understood by an unknown listener. The judgement is based on a short sample of conversational speech, and scoring comprises 5 grades, namely 0 (normal), 1 (different from other children's speech, but not enough to cause comments), 2 (different enough to provoke comments, but still intelligible), 3 (barely understandable to strangers), and 4 (impossible to understand). Hypernasality is associated with an abnormal increase in the nasal resonance of the voice during speech production. The tone of the voice is most perceptible with vowels and voiced consonants. Hypernasality is also scored on a 5-point scale: 0 (absent); 1 (borderline/minimal); 2 (mild); 3 (moderate); and 4 (severe).[10]

The degree of palate movement and the severity of VPI were assessed based on the analysis of the gap size, the evaluation of lateral video images and nasal endoscopy. Accordingly, the most appropriate surgical procedure (Sommerlad–Furlow modified palatoplasty under magnification or modified Furlow palatoplasty with posterior pharyngeal flap) was selected for each patient and performed by the same surgeon. Six months after palatoplasty, the same speech assessment was conducted to evaluate the outcome of the surgery. Since the children were too young to respond by themselves to the HRQoL questionnaire, the parent proxy-report form of PedsQL was used to assess and compare HRQoL in the VCFS patients before and after palatoplasty. The PedsQL is a 23-item self-report questionnaire that measures the core dimensions of health.[16,17] It includes 4 generic core scales: physical functioning (8 items); emotional functioning (5 items); social functioning (5 items); and school functioning (5 items). In addition, 3 summary scores are incorporated into the scoring procedure: the physical health summary score (8 items); the psychosocial health summary score (15 items); and the total scale score (23 items). All dimensions are evaluated using a 5-point response scale: 0 (never); 1 (almost never); 2 (sometimes); 3 (often); and 4 (almost always). The items are reverse-scored so that lower scores indicate better HRQoL.

Statistical analysis

Descriptive statistics were used to express the intelligibility and hypernasality scores of CAPS-A. The Wilcoxon signed-rank test was used to compare the PedsQL generic core scale scores of the VCFS patients before and after palatoplasty. Spearman's non-parametric test was used to evaluate the correlation between speech assessment and HRQoL. Data from the cognitive assessment of speech and the PedsQL questionnaire was analyzed using the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA) and the R software for statistical computing and graphics, v. 3.5.1 (https://www.r-project.org). The intraclass correlation coefficient (*ICC*) was used to evaluate the method error. The statistical significance level was set at $p < 0.05$.

Results

A high level of intra-observer agreement was obtained (*ICC* = 0.98). The participants included 12 boys and 8 girls with a mean age of 42 ±4 months. Of the 20 participants, 6 patients underwent primary Sommerlad–Furlow modified palatoplasty, and 14 patients underwent modified Furlow palatoplasty with posterior pharyngeal flap. The mean total scale HRQoL scores in all domains were significantly lower after the surgery. According to the Wilcoxon test, a significant difference was observed in all dimensions before and after the surgery ($p < 0.05$) (Table 1). The CAPS-A pre- and post-operative results showed that 12 patients (60%) had severe hypernasality (grade 4) before the surgery, which decreased to 3 patients (15%) after the surgery. With regard to speech intelligibility before the surgery, 15 patients (75%) scored 4 (impossible to understand), and 5 (25%) scored 3 (barely understandable to strangers). However, after the surgery, the number of patients in the same categories decreased to 1 (5%) and 4 (20%), respectively. Moreover, 15 patients (75%) achieved adequate speech clarity (grades 0, 1 and 2) after the surgery (Table 2). Spearman's non-parametric test showed no significant correlations between the domains of HRQoL, speech clarity and hypernasality before and after palatoplasty (Table 3).

Table 1. Health Related Quality of Life (HRQoL) before and after the surgery (Wilcoxon signed-rank test)

HRQoL domain	Pre-surgery score	Post-surgery score	*p*-value
Physical functioning	8.5500 ±2.98196	8.1000 ±3.00701	0.007*
Emotional functioning	10.8000 ±2.54641	3.8500 ±1.92696	<0.000*
Social functioning	10.6000 ±2.98064	3.7000 ±2.22663	<0.000*
School functioning	6.3500 ±3.19992	5.5000 ±3.15394	0.011*
All domains	36.3000 ±7.88803	21.1500 ±6.19231	<0.000*

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

Table 2. Intelligibility and hypernasality parameters of the Cleft Audit Protocol for Speech – Augmented (CAPS-A)

Parameter	Grade	Pre-surgery	Post-surgery
Intelligibility	0	–	1 (5)
	1	–	9 (45)
	2	–	5 (25)
	3	5 (25)	4 (20)
	4	15 (75)	1 (5)
Hypernasality	0	–	1 (5)
	1	–	5 (25)
	2	–	8 (40)
	3	8 (40)	3 (15)
	4	12 (60)	3 (15)

Data presented as number (percentage) (*n* (%)).

Table 3. Correlation between the Health Related Quality of Life (HRQoL) domains and the speech parameters (Spearman's test)

HRQoL domain	Intelligibility	Hypernasality
Physical functioning	0.724	0.278
Emotional functioning	0.674	0.248
Social functioning	0.147	0.353
School functioning	0.606	0.896
All domains	0.310	0.916

Discussion

The results of the present study showed significant improvement in the post-operative HRQoL scores across all domains (physical, emotional, social, and school functioning), especially in the emotional and social dimensions ($p < 0.000$). In addition, the speech assessment based on CAPS-A showed improvement in speech intelligibility and hypernasality. There were no significant correlations between HRQoL and the speech parameters. Our findings are in line with the overall findings of previous studies, suggesting that the complexity of multiple affected systems has a compounding effect on QoL.

Varni et al. evaluated the HRQoL of pediatric patients with chronic conditions, such as diabetes, gastrointestinal conditions, cardiac conditions, asthma, and obesity.[19] They found that their patients had a more impaired overall HRQoL than healthy children.[19] Looman et al. reported that children with VCFS had poorer QoL as compared to healthy children and their peers with a single chronic illness (e.g., diabetes, asthma, cardiac anomalies, and cancer).[20] In addition, the results of the Primary Self-Concept Inventory (PSCI) questionnaire showed that patients with cleft lip and palate had lower scores in 2 sub-scales (social and intellectual self-concept), as well as total scores, when compared to patients with cleft lip or cleft palate only.[19]

Interaction between people is complex, and heavily influenced by appearances and visible differences. It involves communication, self-perception, and how one is perceived by others. Although palatal defects in children are not generally life-threatening, they can be distressing to parents and interfere with feeding. Consequently, the defects can decrease a child's physical functioning, which might limit their ability to participate in recreational activities, thus affecting their social and emotional functioning.[21,22] Damiano et al. evaluated factors that influence the HRQoL of preadolescent children with non-syndromic oral clefts.[23] The authors showed that speech and esthetic concerns were important factors affecting HRQoL in children with oral clefts. Considering the effect of palatoplasty on improving the speech and self-confidence of patients, the surgery was recommended as a method to improve speech outcomes, and therefore, the emotional and social dimensions of HRQoL,[23] which is in line with our findings.

Marcusson et al. evaluated the correlation between facial appearance and QoL in patients who received cleft lip and palate treatment during their childhood.[24] They reported that satisfaction with facial appearance was significantly correlated with better QoL and HRQoL, and dissatisfaction with facial appearance was the most significant predictor of depression in both groups.[24] In our study, we observed improved HRQoL among the children, although no alterations in their facial appearance occurred, as they were only cleft palate patients. This indicates that improvement in speech alone had a significantly positive effect on the HRQoL of these syndromic patients.

Speech development is the most troubling consequence of 22qDS for many parents, since the syndrome causes a delay in acquiring the productive language skills, while the receptive skills are near normal.[25] Learning and cognitive disabilities in patients with VCFS may complicate speech development and therapy.[21]

Children with VCFS exhibit both anatomical and physiological abnormalities in the palate and pharynx, which makes the surgical correction of VPI different from that in non-syndromic children with the repaired cleft palate.[25] Wagner et al. evaluated the outcomes of speech surgery in patients with VCFS, and concluded that the surgical management of VPI in such patients was challenging.[8] Unsuccessful primary surgery poses additional challenges to surgeons during revision surgery, e.g., a scarred pterygopalatine fossa (PPF) donor site, a distorted palatal recipient site, and further medialization of the internal carotid arteries.[8] Posterior pharyngeal fat graft surgery is suggested to improve speech function in patients with VPI. However, surgical protocols are contradictory, which complicates the treatment of VCFS patients.[26] In our study, we used 2 different palatal surgical techniques, depending on each patient's needs. Both techniques yielded positive results, with no clear superiority of one over the other. However, due to the complexity of the syndrome, some patients required the continuation of treatment.

Since the syndrome can affect speech mechanisms, phonation can be aberrant due to a laryngeal web, VPI or vocal cord paralysis. In many cases, phonation may remain abnormal to some extent, even after palatoplasty.[27] Losken et al. evaluated sphincter pharyngoplasty for the management of VPI, and concluded that patients with VCFS were more likely to require pharyngoplasty revision.[28] Similarly, in our study, some patients required pharyngoplasty revision and continual speech therapy, despite significant improvement in hypernasality after the surgery. Overall, the surgical treatment of VPI in patients with VCFS is a challenging task due to anatomical, physiological and neurocognitive abnormalities. However, an appropriate surgical procedure can improve the quality of speech in VCFS patients, thus enhancing their QoL and self-esteem.

Limitations

Due to the rare nature of VCFS, the main limitation of the present study was the small number of participants. It is recommended that future studies involve larger sample sizes and more accurate pre- and post-operative speech evaluation procedures, such as nasometry and video nasopharyngoscopy.

Conclusions

Given that children with VCFS face various medical and social problems, physicians can prioritize their therapeutic needs and improve their QoL by choosing a suitable palatal intervention. The satisfactory outcomes of our post-operative speech assessment support recommending palatoplasty to the parents of such patients as a cost-effective procedure. Furthermore, the PedsQL assessment demonstrated that the intervention was beneficial and improved the HRQoL and speech outcomes of these patients.

Ethics approval and consent to participate

The study protocol was approved by the institutional Ethics Committee at Shiraz University of Medical Sciences, Iran (IR.SUMS.REC.1398.535). Written informed consent was obtained from the parents of the patients, and the anonymity of the children was guaranteed.

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 sedative effect of intranasal versus intramuscular ketamine in 2–6-year-old uncooperative dental patients

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Abstract

Background. Conscious sedation has gained more popularity these days, with different routes of drug administration having various advantages and disadvantages. Among all, ketamine is one of the most commonly used drugs in children.

Objectives. The aim of the present study was to compare 2 different routes of ketamine administration – intranasal (IN) vs. intramuscular (IM) – in 2–6-year-old uncooperative children needing dental treatment.

Material and methods. This single-blind, crossover clinical trial was conducted on a group of 26 uncooperative children aged 2–6 years, who required at least 2 similar dental treatment visits. The patients were randomly assigned into 2 groups: group I – IN ketamine at their 1st session and IM ketamine at the 2nd session; and group II – exactly the opposite sequence. The sedative efficacy of the 2 techniques was assessed by 2 independent pediatric dentists based on the Houpt sedation rating scale. The data was analyzed using the Wilcoxon test, the repeated measures analysis of variance (ANOVA) and the least significant difference (LSD) test.

Results. The participants showed reduced crying and movement with improved sleepiness at the 3 time points examined when IM administration was performed as compared to IN sedation ($p < 0.05$). The overall behavior scores were higher for the IM route as compared to the IN route at all tested time points ($p < 0.05$). The operating dentist and the parents believed that the IM route was significantly more effective ($p < 0.05$). The children in the IN session reached equilibrium faster than those in the IM session ($p < 0.05$). No significant statistical differences were noted between the groups with regard to various physiological parameters investigated at different time intervals.

Conclusions. Intramuscular ketamine was more satisfactory and effective than the IN route when sedating uncooperative children for dentistry.

Keywords: intranasal, children, dentistry, sedation, intramuscular

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Introduction

The anxiety-provoking nature of dental treatment and the incomplete development of coping skills in children have turned behavior management problems into one of the most common challenges that pediatric dentists face in their routine practice.[1,2] Dental fear and anxiety are considered one of the main obstacles to successful dental treatment in pediatric dentistry, which can directly affect the quality of dental care.[3] Children aged 3–7 years tend to be more uncooperative,[4] with a decreasing trend based on age.[5]

Both pharmacological and non-pharmacological modes of behavior management have been recommended by the American Academy of Pediatric Dentistry (AAPD).[6] Considering changes in the society and individual attitudes toward traditional physical restraints, the pharmacological behavior management techniques have gained more popularity.[1] Pharmacological management is a broad term that describes the use of drugs to manage the behavior of pediatric patients during dental treatment; it is divided into 2 subcategories – sedation and general anesthesia.[7] Conscious sedation is an advanced behavior management technique indicated in children with low coping capacity, including children with behavior management problems, dental fear and anxiety, mental retardation, or psychiatric conditions.[8]

Various routes, such as oral, intranasal (IN), intramuscular (IM), intravenous (IV), subcutaneous, and inhalation, have been introduced for sedative drug administration.[9] The IN technique is a fast, painless and non-invasive method of drug administration that is occasionally used in pediatric dentistry, especially for young children. The selected medication is sprayed or dripped into the nostrils; it is expected to be absorbed directly into the bloodstream, bypassing the gastrointestinal tract and hepatic metabolism, as in the case of the oral medication delivery route.[1,10] There are reports of a burning sensation following IN administration, which can be minimized by the use of a topical anesthetic spray prior to sedative drug administration.[10] When comparing the sedative effects of different routes, it is important to consider the bioavailability of the drug. The bioavailability of nasally administered ketamine in children has been reported to be approx. 50%, whereas the bioavailability of IM administered ketamine is approx. 93%.[11,12]

Various medications are used for conscious sedation, either as a single drug or in combination to produce synergistic action. Ketamine is a highly effective sedative drug with a broad margin of safety, resulting in a dissociative state, sedation, analgesia, and amnesia, with the preservation of spontaneous respiration and airway reflexes, and without respiratory depression[10] when used at the recommended doses. Subanesthetic doses of ketamine (0.5–1.0 mg/kg) provide sedation and analgesia.[13,14] Ketamine is safe and effective when using the IN route to produce moderate sedation for providing dental care to pediatric dental patients.[15] Nausea and vomiting are common postoperative complications associated with this medication.[16] Postoperative hallucinations have been reported in ketamine cases, leading to its more limited administration, although such side effects occur much less frequently in children.[14]

The present study was conducted to compare IN vs. IM ketamine in a group of 2–6-year-old uncooperative children for dental treatment.

Material and methods

This single-blind, crossover clinical trial was conducted on 26 uncooperative 2–6-year-old children with definitely negative or negative Frankl scores, who were referred to the Pediatric Dentistry Fellowship Clinic at the Dental School of Shahid Beheshti University of Medical Sciences, Tehran, Iran, and required at least 2 similar dental treatment visits. Children with nasal obstruction, respiratory infections, limitations in neck movement, macroglossia, tonsil hypertrophy, micrognathia, or limitations in mouth opening were excluded. The subjects were included if they were classified as ASA I, according to the American Society of Anesthesiology (ASA).

Ethics approval was obtained from the Ethics Committee of the Dental Research Center at Shahid Beheshti University of Medical Sciences (2013-7-ResCom-0011-approved). The parents of the subjects signed a written informed consent form. The nil per os (NPO) period was instructed as no solid foods for 8 h and no clear liquids for 2–3 h prior to sedation.

The children were randomly assigned into one of the 2 groups – group I or group II. Both groups received a midazolam vial (2.5 mg/mL; Dales Pharmaceuticals Ltd., Skipton, UK) at a dosage of 0.5 mg/kg and atropine (0.5 mg/mL; Caspian Tamin Pharmaceutical Co., Tehran, Iran) at a dosage of 0.02 mg/kg, given orally as a pre-medication. The medications were prepared and administered under the direct supervision of the anesthesiologist in charge. All basic information was recorded, including age and weight, along with vital signs.

Group I received midazolam orally as a pre-medication 45 min before 1 mL of 2% lidocaine hydrochloride (ampule 2%; Pasture Institute of Iran, Tehran, Iran) was administered into one of the nostrils by using a syringe to obtain relative analgesia and prevent a burning sensation in the nasal mucosa. Then, after waiting 1 min, a total of 10 mg/kg of ketamine (ampule 50 mg/mL; Rotexmedica, Hamburg, Germany) was administered

into the nostrils with a syringe. Group II received an injection of ketamine into the gluteal muscle at a dosage of 5 mg/kg. All participants were subjected to the other method at their 2nd session.

Upon the onset of sedation and the dissociative effects of ketamine (closed eyes or nystagmus, sleepiness or irresponsiveness to tactile or verbal stimuli, or vague and irrelevant answers[17,18]), supplemental oxygen was administered via a nasal cannula at 2 L/min before the initiation of dental treatment.

Physiological parameters, including the heart rate (HR), blood oxygen saturation (SpO_2), blood pressure (BP), and the respiratory rate (RR), were recorded using a portable monitoring device (Saadat, Tehran, Iran) at baseline, after anesthesia administration, at 15 and 30 min from the start of treatment, and at discharge.

An independent pedodontist judged the level of sedation in all cases at 3 time points – at local anesthesia administration, and at 15 and 30 min from the start of treatment – using the Houpt sedation rating scale (Table 1).[19] The details of the Houpt scale include crying (C), sleep (S), movement (M), and overall behavior (O).

After the completion of treatment, the child was transferred to the recovery room, where constant monitoring was conducted for the next 2 h. The children were discharged when they were judged as responsive to verbal and tactile stimuli. They should have no signs of abnormal breathing while being fully awake, and be able to talk, grasp their hand, or sit and stand. Discharge was declared in the presence of the child's parents and upon the approval of the anesthesiologist.[18]

The potential postoperative side effects, including nausea, fever, restlessness, headache, dizziness, a decrease or an increase in activity, and flushing, were checked for 12 h postoperatively. The parental overall satisfaction was also recorded through a phone conversation 24 h after each session.

Statistical analysis

The data was analyzed using the Wilcoxon test, the repeated measures analysis of variance (ANOVA) and the least significant difference (LSD) test to compare the redundancy of the items with regard to the crossover design of the study.

Table 1. Houpt scale criteria at different time intervals for intranasal (IN) and intramuscular (IM) ketamine administration

Route of drug administration	Sedation score by Houpt et al.[19]	Local anesthesia administration				15 min from the start of treatment				30 min from the start of treatment			
		C	S	M	O	C	S	M	O	C	S	M	O
IN ketamine	1	0 (0.0)	4 (15.4)	0 (0.0)	1 (3.8)	0 (0.0)	6 (23.1)	2 (7.7)	12 (46.2)	0 (0.0)	6 (23.1)	2 (7.7)	14 (53.8)
	2	7 (26.9)	19 (73.1)	12 (46.2)	0 (0.0)	21 (80.8)	19 (73.1)	20 (76.9)	5 (19.2)	22 (84.6)	20 (76.9)	21 (80.8)	7 (26.9)
	3	15 (57.7)	3 (11.5)	11 (42.3)	4 (15.4)	4 (15.4)	1 (3.8)	3 (11.5)	4 (15.4)	4 (15.4)	0 (0.0)	3 (11.5)	1 (3.8)
	4	4 (15.4)	–	3 (11.5)	16 (61.5)	1 (3.8)	–	1 (3.8)	3 (11.5)	0 (0.0)	–	0 (0.0)	4 (15.4)
	5	–	–	–	4 (15.4)	–	–	–	1 (3.8)	–	–	–	0 (0.0)
	6	–	–	–	1 (3.8)	–	–	–	1 (3.8)	–	–	–	0 (0.0)
IM ketamine	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	2	0 (0.0)	6 (23.1)	0 (0.0)	0 (0.0)	3 (11.5)	10 (38.5)	5 (19.2)	0 (0.0)	6 (23.1)	18 (69.2)	12 (46.2)	0 (0.0)
	3	3 (11.5)	20 (76.9)	8 (30.8)	0 (0.0)	5 (19.2)	16 (61.5)	12 (46.2)	2 (7.7)	15 (57.7)	8 (30.8)	12 (46.2)	2 (7.7)
	4	23 (88.5)	–	18 (69.2)	1 (3.8)	18 (69.2)	–	9 (34.6)	4 (15.4)	5 (19.2)	–	2 (7.7)	10 (38.5)
	5	–	–	–	7 (26.9)	–	–	–	10 (38.5)	–	–	–	10 (38.5)
	6	–	–	–	18 (69.2)	–	–	–	10 (38.5)	–	–	–	4 (15.4)

Data presented as number (percentage) (*n* (%)).
Houpt scale criteria: C – crying (1 – hysterical crying; 2 – continuous or strong crying; 3 – intermittent or mild crying; 4 – no crying); S – sleep (1 – fully awake, alert; 2 – drowsy, disoriented; 3 – asleep); M – movement (1 – violent, interrupting; 2 – continuous, making treatment difficult; 3 – controllable, not interfering with treatment; 4 – no movement); O – overall behavior (1 – aborted, no treatment rendered; 2 – poor, treatment interrupted, only partial treatment was completed; 3 – fair, treatment interrupted, but eventually completed; 4 – good, difficult, but all treatment was performed; 5 – very good, some limited crying or movement; 6 – excellent, no crying or movement).

Results

The data was collected from 26 children (15 boys and 11 girls) aged 2–6 years, with a mean age of 39.8 months and a mean weight of 14.6 kg. In total, 15 patients (57.7%) scored definitely negative, and 11 (42.3%) scored negative according to the Frankl's behavior rating scale (FBRS).[7]

According to the Wilcoxon signed-rank test, the child's acceptance rate of the IN route was higher as compared to the IM route. Five children took the drug reluctantly, but without resistance, through the IN route, while all children resisted taking the medicine via the IM route.

The drug onset time was 5–10 min and its action lasted for 35–60 min (48.3 min on average) for the IN route, while for the IM route, the duration was 5–20 min (13.2 min on average) and 60–90 min, respectively, with the difference being statistically significant ($p < 0.05$).

According to the Wilcoxon signed-rank test, the Houpt scale scores were significantly different between the 2 groups at the time of administration, and after the first and second 15 min. The children cried less, had a deeper sleep and showed fewer movements in the case of the IM route as compared to the IN route at all 3 time points ($p < 0.05$) (Tables 1 and 2).

For the IN route, the most frequent scores with regard to the overall behavior were 4 (good) at the time of administration, and 1 (aborted) at 15 min and 30 min from the start of treatment. For the IM route, the most frequent overall behavior scores were 6 (excellent) at administration, 5/6 (very good/excellent) after the first 15 min and 4/5 (good/very good) after another 15 min. The Wilcoxon test showed statistically significant differences between the 3 time points. The success rate in terms of sedation and ease of treatment was higher in the IM session as compared to the IN session at all 3 time points ($p < 0.05$) (Tables 1 and 2).

In addition, the operating dentist believed that the IM route was significantly more effective than the IN route ($p < 0.05$). The parents also preferred the IM route and believed that it was much more effective ($p < 0.05$). The children in the IN session reached equilibrium (the discharge status with independent normal walking) faster than those in the IM session, and the difference was statistically significant ($p < 0.05$).

The side effects of ketamine included flushing, abnormal muscle tone and emergence reactions in both groups during treatment and before discharge, with the differences between the groups not reaching significance ($p > 0.05$) (Table 3). The most commonly reported post-treatment complications were vomiting and flushing in both groups; 12 patients in the IN session and 11 patients in the IM session reported some sort of vomiting post-operatively, with the difference between the groups being non-significant ($p = 0.317$). Five patients in each group showed signs of flushing, which was not statistically significant ($p > 0.05$) (Table 3). Visual disturbances, desaturation and behavioral abnormalities were not observed in either of the groups.

Considering the overall behavior scores, 4, 5 and 6 on the Houpt scale indicate successful sedation. The Wilcoxon test showed no significant difference in the success rate of the 2 different routes at the time of drug administration, while after the first and second 15 min, the IM route was statistically more effective ($p < 0.05$) (Table 2).

The repeated measures ANOVA and the LSD test showed that the differences in physiological parameters (HR, SpO_2, BP, and RR) were not statistically significant between the groups and within the groups. The mean HR in each group was at its lowest at baseline and increased insignificantly in the IN session at the subsequent time points, while the changes in the IM session were significant. There were no significant differences in HR between the 2 groups at baseline, after anesthesia administration, 15 min after starting the treatment, and at discharge, while HR was significantly higher in the IM session 30 min after starting the treatment.

Table 2. Successful sedation at different time intervals for intranasal (IN) and intramuscular (IM) ketamine administration (Wilcoxon test)

Route of drug administration	Local anesthesia administration	15 min from the start of treatment	30 min from the start of treatment	Wilcoxon test (child acceptance)	Wilcoxon test (onset time)	Wilcoxon test (overall behavior)
IN ketamine	21 (80.8)	5 (19.2)	4 (15.4)	$p > 0.05$	$p < 0.05$	$p < 0.05$
IM ketamine	26 (100.0)	24 (92.3)	24 (92.3)			

Data presented as *n* (%).

Table 3. Complications and side effects of intranasal (IN) and intramuscular (IM) ketamine administration

Route of drug administration	During treatment and before discharge				Post-treatment		
	flushing	abnormal muscle tone	emergence reactions	no complications	no vomiting	vomiting once	vomiting twice
IN ketamine	5 (19.2)	4 (15.4)	4 (15.4)	13 (50.0)	14 (53.8)	10 (38.5)	2 (7.7)
IM ketamine	5 (19.2)	3 (11.5)	5 (19.2)	13 (50.0)	15 (57.7)	11 (42.3)	0 (0.0)

Data presented as *n* (%).

Discussion

Since different routes of drug administration may influence the sedative properties of drugs, this study was conducted to compare IN vs. IM ketamine in 2–6-year-old uncooperative children while receiving dental services. Various medications and methods have been tested to overcome a child's interfering behaviors in the dental office. Based on the results of this study, it can be stated that the IM administration of selected sedative agents, despite a child's resistance during injection, provides more efficient sedation according to the Houpt scale.

Intranasal ketamine has gained popularity due to the ease of administration, minimal distress, the reduced risk of needle-stick injuries, and the reduced need for skills to establish IV access.[20] Due to its acidic pH, ketamine may cause some discomfort, and the irritation and inflammation of the nasal mucosa, which may last for up to 1 h. In the current study, 2% lidocaine hydrochloride was instilled into the nasal cavities before the administration of ketamine to anesthetize the nasal mucosa, and thus reduce discomfort and irritation.[21]

The results of the current study demonstrated that the average onset time of the drug was 13 min for the IM route and 8 min for the IN route. When using the IN route, the drug directly enters the highly vascularized nasal mucosa and the systemic circulation. It appears that when the medication is administered IN, dropwise with a syringe, some of it enters the oropharyngeal area and is swallowed, so the amount of drug that is directly absorbed by the mucosa may be significantly reduced, resulting in a longer drug onset time in certain instances. Using the Mucosal Atomization Device (MAD®) for spraying the drug into the nasal mucosa significantly reduces the amount of drug entering the mouth, and accelerates the absorption and effect of the drug.[22,23] Tsze et al. reported a sedation onset interval of 3–9 min with the nasal administration of 9 mg/kg of ketamine, using MAD.[24] In a study conducted by Shashikiran et al., the onset and recovery time of IN midazolam was reported to be shorter than in the case of the IM route.[25] Al-Rakaf et al. also reported the onset of sedation at about 8–15 min after the administration of IN midazolam,[26] which is less than the time recorded in the current study. In both of the abovementioned studies, midazolam was administered with a nasal atomizer.[25,26] Before administering the drug into the nasal mucosa, the nose should be briefly examined for abnormalities and excessive nasal secretions, which may affect the amount of drug absorbed.[27]

The results of this investigation demonstrated that out of 26 patients, only 9 children had sufficient sedation at 15 min and 30 min when using IN ketamine (Table 2). All the subjects receiving IM ketamine had their treatment completed in less than 30 min, which can be justified by the fact that IM ketamine sedation has a more profound effect while in action. The droplet delivery of the medication to the nose may result in a speedy run into the oropharengeal space before having a chance to be absorbed by the nasal mucosa, thus resulting in a lower uptake and a lesser effect of the drug. Therefore, the blood drug level and the level of sedation are directly affected if atomizers are not used.[28]

Shashikiran et al. demonstrated that both IM and IN midazolam were successful, and stated that the same profile could be drawn for IM and IN midazolam in terms of effects and safety,[25] while Lam et al. claimed that the sedation induced by IM midazolam was deeper as compared to IN midazolam – children in the IM group showed less movement and better overall behavior according to the Houpt scale; however, no significant differences were reported with regard to children's crying during that investigation.[28]

According to the results of this study, the parents believed that the IM route was more effective than the IN route. They were more satisfied with IM administration, even though the children showed less resistance to receiving the drug via the IN route. The parents expressed greater satisfaction with the time needed for their children to reach equilibrium for discharge for the IN route, as it was shorter than for the IM route. Of note, the swallowed amount of the drug which undergoes the first-pass metabolism can provide a prolonged effect in certain instances.[17]

Nausea and vomiting are the common side effects of ketamine, being more prevalent in adults in comparison with children.[17] In the current study, vomiting and flushing were the 2 most common side effects reported by the parents after discharge. The incidence of vomiting was lesser than that of flushing, with no differences between the groups. An increased body temperature or flushing are the side effects of the atropine use, even at the minimum recommended dose of 0.02 mg/kg.[17] The period of preoperative fasting and dehydration could also play a role in the incidence of flushing. Intravenous liquid was injected during the treatment to compensate. Excessive muscle contractions in the arms and legs were also observed in a few children in both groups as the side effects of ketamine. The concurrent use of benzodiazepines, such as midazolam, with ketamine, has been shown to be helpful in decreasing the incidence of these conditions.[17,18]

There were no episodes of desaturation, behavioral disorders, hallucinations, or visual disturbances observed in children during this investigation. Diaz did not report any symptoms of nausea or vomiting after the administration of IN ketamine in comparison with placebo.[29] Bahetwar et al. stated that nausea and vomiting were noticed only in a small number of patients, while no statistically significant differences were reported between the groups.[15]

In the current study, the evaluation of physiological parameters (SpO_2, BP and RR) did not reveal any significant differences between the groups at different time intervals, which is consistent with previous studies.[1,30]

Ketamine can cause BP to increase by about 20–25% above the pre-drug levels; therefore, it is recommended to use benzodiazepines along with ketamine to compensate for such a rise and an increased HR. It should be noted that ketamine can cause respiratory apnea at higher doses.[17]

An increase in HR was observed in both groups after the administration of local anesthesia. This increase may be due to the effects of ketamine or the epinephrine content in lidocaine. On the other hand, atropine, along with a sedative medication, can also play a role in increasing HR. Pain during the injection or dental treatment is also considered a contributing factor for an HR increase.

Conclusions

The IN route had a relatively acceptable sedation level for short treatment sessions (less than 10–15 min). The IM route provided more reliable sedation for treatment procedures longer than 10–15 min.

The parents preferred the IM route due to less discomfort of the patient.

In comparison with the IN route, IM drug administration is preferred in terms of inducing better sedation for the successful completion of treatment and causing the least amount of discomfort for the patient, as well as the stability of the cardiovascular and respiratory systems.

Ethics approval and consent to participate

Ethics approval was obtained from the Ethics Committee of the Dental Research Center at Shahid Beheshti University of Medical Sciences, Tehran, Iran (2013-7-ResCom-0011-approved). The parents of the children signed a written informed consent form.

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

Sleep architecture and vitamin D in hypertensives with obstructive sleep apnea: A polysomnographic study

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Abstract

Background. Obstructive sleep apnea (OSA) and arterial hypertension (AH) are closely linked disorders with common pathophysiological features.

Objectives. The present study aimed to investigate the relationship between AH and OSA by examining sleep architecture, vitamin D concentration and electrolyte levels in patients with these coexisting conditions.

Material and methods. A total of 133 patients suspected of having OSA were recruited for examination. The participants were divided into 2 groups: hypertensives ($n = 52$); and normotensives ($n = 81$). One full-night polysomnographic examinations were conducted, followed by the statistical analysis of the collected data.

Results. Hypertensive individuals displayed increased apnea–hypopnea index (AHI), oxygen desaturation index (ODI), respiratory arousal index (RAI), and periodic limb movement index (PLMI) as compared to non-hypertensive individuals. Moreover, sleep efficiency (SE), the bruxism arousal index (BAI) and oxygen saturation (SpO_2) level were decreased in the hypertensive group. In terms of biochemical parameters, hypertensive individuals exhibited a lower magnesium (Mg) level, and higher levels of C-reactive protein (CRP), uric acid (UA) and glucose. Notably, there were no statistical differences in vitamin D concentration between hypertensive and normotensive individuals.

Conclusions. The study explored the potential influence of calcium (Ca), Mg, vitamin D, and UA concentrations on the sleep architecture of patients with comorbid AH and OSA. The findings revealed several notable associations. Firstly, sleep fragmentation correlated with Ca level, suggesting a potential role for both Ca and vitamin D in sleep arousals. Secondly, a higher UA concentration was linked to a higher AHI and increased sleep fragmentation. Additionally, alterations in Mg concentration were observed among hypertensive individuals with OSA. However, further research is needed to fully comprehend the potential impact of these factors on the sleep architecture of hypertensive individuals with apnea.

Keywords: uric acid, vitamin D, obstructive sleep apnea, hypertension, periodic limb movements of sleep

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Introduction

Obstructive sleep apnea (OSA) is a highly prevalent sleep disorder that, based on rough estimates, may potentially impact nearly one billion adults globally, specifically those aged between 30 and 69 years. Within this population, approx. 425 million individuals are estimated to have moderate to severe OSA that requires medical intervention.[1] Patients with OSA commonly experience symptoms such as excessive daytime sleepiness, loud snoring, and witnessed pauses during breathing or awakenings from gasping for air after sleep events.[2] The signs of OSA include the repetitive collapse of the upper airway,[3] sleep fragmentation,[4] hypoxemia,[5] hypercapnia,[6] and increased sympathetic activity.[7] Furthermore, individuals with OSA may experience morning headaches, a dry mouth or a sore throat upon waking, difficulty with concentrating, and irritability.[8] The American Academy of Sleep Medicine (AASM) defines polysomnography (PSG) as the gold standard for diagnosing OSA.[9] Obstructive sleep apnea is associated with intermittent airflow obstruction leading to periods of a decreased oxygen level in the bloodstream.[10] These fluctuations in oxygen can have detrimental impact on various organs and systems, particularly the cardiovascular system. As a result, OSA has been associated with an increased risk of arterial hypertension (AH), heart disease, stroke, and other cardiovascular complications.[11]

Arterial hypertension, as defined by the American Heart Association (AHA) and other major guidelines, refers to a patient having a systolic blood pressure of 140 mmHg or higher and/or a diastolic blood pressure of 90 mmHg or higher upon repeated examination.[12] Arterial hypertension pathophysiology shares similarities with OSA, including the evidence of sympathetic nervous system activation[13] due to peripheral resistance,[14] renin–angiotensin system (RAS) involvement[15] and endothelial dysfunction.[16] Numerous studies have recognized an excessive sodium (Na) intake as a risk factor for hypertension, as it disrupts the balance of microelements in cellular fluids, leading to the contraction of vascular smooth muscles, a reduced blood flow and an increased blood pressure.[17] Besides changes in macroelements (Na and potassium (K)), studies have also shown plasma vitamin D alterations in hypertensive patients. Approximately 50% of the global population is affected by vitamin D insufficiency despite lifestyle and environmental factors.[18] Lower levels of vitamin D are associated with an increased risk of cardiovascular events, possibly due to its influence on blood pressure regulation.[19] One potential connection is the inverse association between serum vitamin D level and the renin–angiotensin–aldosterone system (RAAS) in patients with AH.[20,21] Furthermore, vitamin D plays a role in maintaining calcium (Ca) homeostasis through parathyroid hormone (PTH), and some studies have reported an association between PTH and hypertension.[22–24] Moreover, vitamin D has vasodilatory and antiatherosclerotic properties, influencing the vessel wall.[25,26] However, it is important to note that some research suggests that vitamin D may increase vascular resistance by enhancing sensitivity to vasoconstrictors.[27–29]

Studies have revealed that, mostly due to the common pathomechanism in AH and OSA, approx. 30–40% of hypertensive patients also have OSA, while 50% of individuals with OSA have a history of AH.[30,31] Furthermore, OSA frequently coexists in patients with resistant hypertension,[32] which enhances the connection between these conditions. The primary pathophysiological mechanisms implicated in the changes induced by OSA include intermittent episodes of hypoxemia and reoxygenation, frequent arousals, and alterations in intrathoracic pressure.[33] These factors contribute to the development and progression of AH in individuals with OSA. The increasing prevalence of sleep disorders in the general population, particularly OSA, specifically among obese and hypertensive individuals, and those resistant to antihypertensive therapy, highlights the importance of implementing effective screening, diagnosis and treatment strategies for OSA to mitigate cardiovascular risks.

Patients with both OSA and AH are indeed at a heightened cardiovascular risk. Therefore, it is crucial to recognize all factors, including polysomnographic and biochemical markers, that can influence the effectiveness of sleep and contribute to a cardiovascular risk. Understanding the common pathomechanisms connecting sleep disorders, particularly OSA, and hypertension is essential for understanding the complex interplay between these conditions. By implementing comprehensive screening, diagnosis and treatment strategies, including PSG, and considering biochemical markers, healthcare professionals can effectively manage sleep disorders and hypertension. Such an approach mitigates associated cardiovascular risks, and ultimately improves overall patient outcomes.

The present study aimed to investigate the sleep architecture and vitamin D concentration in patients with coexisting AH and OSA as compared to normotensives. Furthermore, we aimed to assess the levels of electrolytes and explore their relationship with sleep parameters. By examining these factors, we sought to gain a comprehensive understanding of the interplay between AH, OSA and electrolyte levels, which could contribute to improving management strategies and reducing risks for affected individuals.

Material and methods

This prospective observational study involving 133 patients was conducted at the University Hospital in Wroclaw, Poland. The study was approved by the Wroclaw Medical University Bioethical Committee (No ID KB-407/2022) and adhered to the principles outlined in the Declaration

of Helsinki. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist (a combined checklist for cohort, case–control and cross-sectional studies) to ensure the transparency and comprehensiveness of the findings.[34] Before participation, all patients provided written informed consent, thereby affirming their comprehension of the objectives and procedures of the study, as well as potential risks and benefits.

Participants

The patients included in the study were referred to the Sleep Laboratory of the Department and Clinic of Internal Medicine, Occupational Diseases, Hypertension, and Clinical Oncology at Wroclaw Medical University, Poland. Eligible participants were aged 18 years and above and had suspected OSA. The study cohort comprised individuals of Caucasian ethnicity with an average age of 47.8 ±16.6 years who willingly agreed to participate in the examination. The exclusion criteria were established to ensure the validity of the study; they comprised severe mental disorders, active malignancy, active inflammation, secondary hypertension, recent myocardial infarction or stroke within 6 weeks, an estimated glomerular filtration rate (eGFR) of less than 30 mL/min, pregnancy, vitamin D supplementation in the past 6 months, and an inability to undergo a polysomnographic examination (Fig. 1). The characteristics of the study group are presented in Table 1. The patients were stratified into 2 groups – one consisting of patients diagnosed with hypertension (n = 52) and the other including patients without hypertension (n = 81). The hypertension diagnosis followed the guidelines provided by the European Society of Cardiology and the European Society of Hypertension (ESC/ESH).[35] In addition, a subgroup of normotensive individuals without sleep disorders (n = 34) was extracted for further analysis.

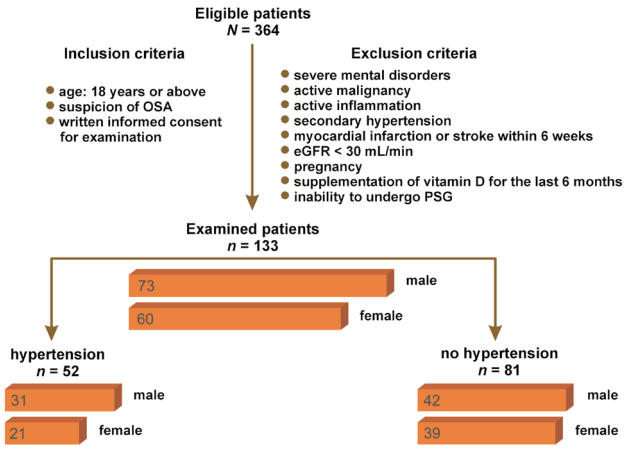

Fig. 1. Study inclusion and exclusion criteria

OSA – obstructive sleep apnea; eGFR – estimated glomerular filtration rate; PSG – polysomnography.

Table 1. Characteristics of the entire study group (N = 133)

Characteristics		Statistics
Comorbidities *n* (%)	AH	52 (39.1)
	prior myocardial infarction	7 (5.3)
	prior stroke	6 (4.5)
	diabetes	24 (18.0)
	ischemic heart disease	10 (7.5)
PSG parameters *M* ±*SD*	AHI	19.83 ±23.42
	ODI	18.21 ±21.25
	snore [%]	20.82 ±21.11
	PLMI	8.02 ±17.24
	SL [min]	15.16 ±11.17
	REM latency [min]	101.01 ±77.81
	WASO [min]	65.83 ±61.39
	SE [%]	81.46 ±14.30
	N1 [%]	6.17 ±4.87
	N2 [%]	48.61 ±18.33
	N3 [%]	26.19 ±24.81
	REM [%]	22.58 ±9.72
	av SpO_2 [%]	92.73 ±4.34
	min SpO_2 [%]	82.16 ±8.87
	SpO_2 < 90% duration [%]	10.11 ±16.30

Data presented as number (percentage) (n (%)) or as mean ± standard deviation (M ±SD). AH – arterial hypertension; AHI – apnea–hypopnea index; ODI – oxygen desaturation index; snore [%] – snore percentage during sleep; PLMI – periodic limb movement index; SL – sleep latency; REM latency – rapid eye movement sleep latency; WASO – wakefulness after sleep onset; SE – sleep efficiency; N1 – sleep stage 1; N2 – sleep stage 2; N3 – sleep stage 3; REM [%] – REM sleep stage percentage of the total sleep time; av SpO_2 – average oxygen saturation; min SpO_2 – minimal oxygen saturation; SpO_2 < 90% duration [%] – percentage of the average oxygen saturation below 90%.

To determine the minimum required sample size, a sample size calculator (https://www.calculator.net/sample-size-calculator.html) was utilized. The parameters used for the calculation included a population size of 3,000,000 (representing the population size of the Lower Silesian Voivodeship), a confidence level of 95% (default value), a maximum error of 5%, and a population proportion of 8%. Based on these calculations, a minimum sample size of 114 individuals was determined and achieved for the study.

During the admission of the participants to the Sleep Laboratory, blood samples were collected from all individuals into polyethylene terephthalate plastic tubes containing K2EDTA (Becton, Dickinson and Company, Franklin Lakes, USA) via venipuncture. After collection, the samples were stored at –70°C until they were ready for analysis. All blood and urine samples were analyzed at the Main Laboratory of Wroclaw Medical University, following the standard laboratory protocols of the Wroclaw Medical University Teaching Hospital. The plasma concentrations of C-reactive protein (CRP) [mg/dL], Na [mmol/L], K [mmol/L], Ca [mg/dL], magnesium (Mg) [mg/dL], and vitamin D [ng/mL] were determined using appropriate laboratory techniques.

Polysomnography

The polysomnograms were analyzed using the Nox A1 Noxturnal system (Nox Medical, Reykjavik, Iceland). The recorded data was divided into 30-second epochs and scored according to the sleep scoring criteria outlined in the AASM Manual for the Scoring of Sleep and Associated Events, v. 2.6.[36] Various physiological signals were recorded during the study by using numerous techniques and devices, including electroencephalography (EEG) to measure brain activity, electrooculography (EOG) to detect eye movements, electromyography (EMG) to assess muscular tension (recorded from the chin and tibial electrodes), a nasal pressure sensor to measure airflow, and inductive plethysmography to evaluate chest and abdomen movements. In addition to these recorded signals, the patient's body position and bilateral masseter EMG were also captured. Episodes of bruxism were classified according to the AASM standards, distinguishing between phasic, tonic and mixed forms. Arousals were categorized as spontaneous, respiratory, bruxism, and periodic limb movement (PLM) arousals. The manual scoring and the analysis of the collected data were conducted by a qualified physician (H.M.) from the Sleep Laboratory of Wroclaw Medical University, following the guidelines provided by AASM.

Statistical analysis

Statistical analysis employed Dell™ Statistica™, v. 13 (Dell Inc., Austin, USA). Descriptive statistics, including the mean (*M*), standard deviation (*SD*) and range (minimum and maximum) values, were calculated for the estimated parameters in the study groups. Correlation analysis was conducted to identify any associations between the variables of interest. Statistical significance was determined based on a two-sided *p*-value of less than 0.05.

Results

The study participants were divided into 2 groups – hypertensive and normotensive – and comparisons were made between them. Several PSG parameters exhibited statistically significant differences between the 2 groups (Table 2). Among hypertensive patients, the apnea–hypopnea index (AHI) was significantly higher (28.87 ±26.23 vs. 13.95 ±19.41; $p < 0.05$) as compared to the non-hypertensive group. Similarly, the oxygen desaturation index (ODI) was higher in the hypertensive group (27.39 ±24.27 vs. 12.24 ±16.64; $p < 0.05$). The periodic limb movement index (PLMI) and the snore percentage were also significantly higher among patients with hypertension (11.74 ±24.88 vs. 5.61 ±8.84; $p < 0.05$, and 31.12 ±22.44% vs. 14.13 ±17.29%; $p < 0.05$, respectively). Differences were also observed in the distribution of sleep stages, with a significantly lower duration of rapid eye movement (REM) sleep among hypertensive patients (20.58 ±8.73% vs. 23.89 ±10.15%; $p < 0.05$).

Table 2. Polysomnography parameters of the examined groups

Parameter	Hypertension	No hypertension	*p*-value
AHI	28.87 ±26.23	13.95 ±19.41	<0.05*
ODI	27.39 ±24.27	12.24 ±16.64	<0.05*
Snore [%]	31.12 ±22.44	14.13 ±17.29	<0.05*
PLMI	11.74 ±24.88	5.61 ±8.84	<0.05*
SL [min]	21.45 ±28.86	15.98 ±13.82	0.77
REM latency [min]	99.13 ±80.02	102.21 ±76.85	0.78
WASO [min]	74.30 ±65.79	60.32 ±58.11	0.09
SE [%]	78.36 ±16.50	83.48 ±12.36	<0.05*
N1 [%]	7.62 ±6.61	6.27 ±6.09	0.26
N2 [%]	47.27 ±12.27	51.21 ±26.5	0.96
N3 [%]	24.53 ±8.64	27.26 ±31.13	0.40
REM [%]	20.58 ±8.73	23.89 ±10.15	<0.05*
AI	8.28 ±7.82	6.59 ±5.77	0.34
RAI	4.90 ±7.80	2.42 ±5.31	<0.05*
RERAs	0.44 ±0.69	0.22 ±0.38	<0.05*
BAI	0.61 ±0.85	1.11 ±1.28	<0.05*
PLMAI	0.12 ±0.29	0.19 ±0.55	0.88
SAI	2.21 ±1.65	2.58 ±2.25	0.46
BEI	4.23 ±3.99	4.10 ±3.41	0.83
RDI	29.47 ±26.03	13.19 ±18.06	<0.05*
Apneas/h	15.49 ±20.25	6.72 ±12.85	<0.05*
Hypopneas/h	13.39 ±11.85	7.34 ±11.34	<0.05*
av SpO_2 [%]	91.82 ±2.18	93.33 ±5.22	<0.05*
min SpO_2 [%]	79.81 ±9.07	83.69 ±8.45	<0.05*
SpO_2 < 90% duration [%]	17.20 ±20.14	5.50 ±11.15	<0.05*

Data presented as *M* ±*SD*. AI – arousal index; RAI – respiratory arousal index; RERAs – respiratory effort-related arousals; BAI – bruxism arousal index; PLMAI – periodic limb movement arousal index; SAI – spontaneous arousal index; BEI – bruxism episode index; RDI – respiratory disturbance index; * statistically significant.

Sleep efficiency (SE) was reduced in the hypertensive group as compared to the non-hypertensive group (78.36 ±16.50% vs. 83.48 ±12.36%; $p < 0.05$). Moreover, the average oxygen saturation (SpO_2) level was lower in the hypertensive group as compared to the normotensive group (91.82 ±2.18% vs. 93.33 ±5.22%; $p < 0.05$), while the minimal SpO_2 level was also significantly lower in the hypertensive group (79.81 ±9.07% vs. 83.69 ±8.45%; $p < 0.05$). Additionally, the time spent with SpO_2 below 90% was significantly higher in patients with hypertension (17.20 ±20.14% vs. 5.50 ±11.15%; $p < 0.05$), as shown in Table 2.

The examination of electrolyte concentration levels revealed several significant differences between the hypertensive and non-hypertensive groups (Table 3). Among hypertensive patients, there was a decrease in Mg concentration (1.87 ±0.24 mg/dL) as compared to non-hypertensive individuals (2.01 ±0.16 mg/dL) ($p < 0.05$). Vitamin D concentration in the hypertensive group (30.24 ±11.07 ng/mL)

Table 3. Blood test parameters among the examined patients

Parameter	Hypertension	No hypertension	*p*-value
CRP [mg/dL]	2.78 ±2.58	2.46 ±5.00	<0.05*
ESR [mm/h]	10.97 ±12.13	6.58 ±5.54	<0.05*
UA [mg/dL]	5.67 ±1.26	5.07 ±1.42	<0.05*
Glucose [mg/dL]	117.94 ±39.12	97.27 ±20.12	<0.05*
Na [mmol/L]	139.69 ±2.22	140.07 ±2.00	0.69
K [mmol/L]	4.27 ±0.34	4.31 ±0.28	0.38
Ca [mg/dL]	9.34 ±0.29	9.32 ±0.32	0.53
Mg [mg/dL]	1.87 ±0.24	2.01 ±0.16	<0.05*
Vitamin D [ng/mL]	30.24 ±11.07	34.17 ±18.05	0.40

Data presented as *M* ±*SD*. CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; UA – uric acid; Na – sodium; K – potassium; Ca – calcium; Mg – magnesium. * statistically significant.

was similar to that in normotensive individuals (34.17 ±18.05 ng/mL) ($p > 0.05$). Interestingly, when comparing vitamin D levels between hypertensive and normotensive individuals without sleep disorders, a significant difference was observed (30.24 ±11.07 ng/mL vs. 39.42 ±24.70 ng/mL; $p < 0.05$) (Fig. 2). Additionally, hypertensive patients exhibited a higher level of CRP (2.78 ±2.58 mg/dL) as compared to non-hypertensive individuals (2.46 ±5.00 ng/mL) ($p < 0.05$). The erythrocyte sedimentation rate (ESR) was also higher in the hypertensive group (10.97 ±12.13 mm/h) than in the non-hypertensive group (6.58 ±5.54 mm/h) ($p < 0.05$). Furthermore, uric acid (UA) concentration was elevated in hypertensive individuals (5.67 ±1.26 mg/dL) as compared to non-hypertensive individuals (5.07 ±1.42 mg/dL) ($p < 0.05$) (Table 3).

In this study, we observed significant correlations between blood and sleep parameters. There was a negative correlation between Mg concentration and AHI ($r = -0.25$; $p < 0.05$), indicating that a lower Mg level was associated with a higher severity of sleep apnea. Similarly, we observed a negative correlation between Mg concentration and ODI ($r = -0.26$; $p < 0.05$). Furthermore, we found a negative correlation between Ca concentration and the arousal index (AI) ($r = -0.26$; $p < 0.05$), as well as correlations between UA concentration and AHI ($r = 0.27$; $p < 0.05$), ODI ($r = 0.28$; $p < 0.05$) and the average SpO_2 ($r = -3.34$; $p < 0.05$). Additionally, there were correlations between vitamin D concentration and AHI ($r = -0.18$; $p > 0.05$) and the average SpO_2 ($r = 0.21$; $p < 0.05$).

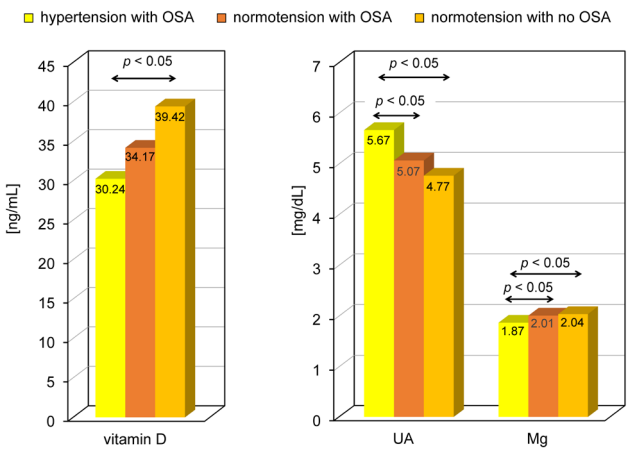

Fig. 2. Statistical differences in blood test parameters among the examined patients

Discussion

The prevalence of AH in individuals with OSA is significantly higher in comparison with the healthy population.[37] In this study, we aimed to compare sleep architecture parameters, including sleep fragmentation, inflammatory and metabolic markers, and vitamin D concentration, between hypertensive and normotensive individuals with OSA. Additionally, we aimed to assess different types of arousal in hypertensives in comparison with normotensives to better understand sleep fragmentation as a novel cardiovascular risk factor.

Our findings revealed increased respiratory parameters (AHI, respiratory effort-related arousals (RERAs) and snoring) and altered SpO_2 parameters (ODI, average SpO_2 and minimal SpO_2) in hypertensive individuals with OSA, which is consistent with previous studies.[38] Furthermore, we observed sleep architecture differences between hypertensive and normotensive individuals.

Sleep architecture

Sleep architecture consists of 2 main stages: REM sleep; and non-REM sleep. In hypertensive individuals with OSA, we found a decrease in REM sleep duration, while non-REM sleep duration was similar in both groups. Rapid eye movement sleep is characterized by REMs, low-voltage EEG[39,40] and muscle atonia.[41] It is also associated with the occurrence of dreams, as discovered by Aserinsky and Kleitman.[42] A decrease in REM sleep duration accompanied by longer N1 sleep duration has been previously observed in OSA patients,[43] which aligns with our results. Additionally, it has been observed that OSA patients experience a decrease in both REM sleep and slow-wave sleep (SWS) duration.[44] However, a recent study reported no changes in sleep architecture and the total sleep time (TST) in hypertensive individuals with OSA,[45] which partially aligns with our results of similar TST duration in hypertensives and normotensives.

Notably, this study contributes by showing, for the first time, a decrease in REM sleep duration among hypertensive individuals with OSA. Sleep architecture studies often yield inconsistent findings or contradict the existing literature regarding hypertension. Recently, Ren et al. showed that decreased SWS duration was associated with higher odds of hypertension in OSA, but not in primary snoring, although they did not investigate the relationship between REM sleep and hypertension.[46]

The function of REM sleep is complex and not fully understood, though it is widely recognized as a crucial period for memory consolidation and emotional processing.[47] One hypothesis suggests that REM sleep may serve as a "gate" for wakefulness,[48] as most spontaneous awakenings occur during REM sleep.[49,50] Therefore, the observed reduction in REM sleep among hypertensives with OSA may be a defensive response to increased sleep fragmentation. However, further research is needed to explore this hypothesis.

The present study also revealed decreased SE and increased wakefulness after sleep onset (WASO) duration in hypertensive individuals, indicating poor sleep quality. We further investigated the specific causes of arousals by assessing different types of arousal. Respiratory arousals were the most commonly observed in both groups, though hypertensives exhibited more respiratory arousals than normotensives, highlighting sleep fragmentation. There were no statistically significant differences in spontaneous and PLM arousals between the study groups.

Interestingly, normotensive individuals experienced a higher incidence of arousals related to bruxism episodes despite a similar bruxism episode index (BEI) in the hypertensive and normotensive groups. Previous studies have suggested that individuals with mild apnea experience more bruxism events than those with severe apnea.[51] However, non-apneic hypertensives have been found to exhibit more bruxism episodes than normotensives.[52] The occurrence of bruxism episodes can be influenced by various factors, such as age, OSA severity, arousals, sympathetic activity, and hypertension, which may explain why BEI was similar in hypertensives and normotensives in this study. Our findings suggest that in hypertensive individuals, bruxism episodes may not commonly lead to arousals, unlike in normotensive individuals.

The most prevalent type of arousal observed in this study was respiratory arousal. Recent studies associated respiratory events with poor sleep quality, excessive daytime sleepiness and an increased cardiometabolic risk.[53] Although arousal scoring is not mandatory according to the AASM guidelines, omitting arousals may result in failing to identify patients who could benefit from OSA treatment. These patients, typically female, younger and non-obese, may experience respiratory events causing sleep fragmentation, even without significant oxygen desaturation.[54] Recurrent arousals result in sympathetic activation, inflammation, oxidative stress, and metabolic dysfunction, leading to cardiometabolic disturbances and cardiovascular diseases.[55] Animals exposed to long-term sleep fragmentation for 12 weeks demonstrated the initiation of mild hypertension, endothelial dysfunction and early structural vascular changes.[56] Moreover, repetitive arousals have been associated with repetitive blood pressure rises,[57,58] indicating that sleep fragmentation may contribute to AH. Interestingly, it has been observed that only respiratory arousals lead to blood pressure surges.[59]

Notably, respiratory arousals were the most common type of arousal observed in this study. To the best of our knowledge, we have demonstrated, for the first time, the significance of different types of arousal in AH among individuals with OSA.

Movement disorders

Limited data is available on the relationship between the PLM disorder (periodic limb movements of sleep – PLMS) and AH. However, a recent meta-analysis indicated an increased risk of hypertension among individuals with PLMS.[60] While PLMS is often associated with OSA, the current criteria set by AASM do not score limb movements if they occur within 0.5 s before or after a respiratory event. A recent study demonstrated a strong correlation between PLMS and a lower threshold for a respiratory arousal.[61] The analysis of the European Sleep Apnea Database (ESADA) revealed an association between PLMS and an increase in systolic blood pressure, regardless of AHI, and independent of other clinical and sociodemographic factors.[62] The authors concluded that individuals with a PLMS phenotype might be at an increased risk of cardiovascular diseases in the presence of OSA.[62]

Consistent with previous findings, this study also found a higher prevalence of PLMS in hypertensive individuals as compared to normotensive individuals. However, a retrospective hospital-based study in a Korean population found no association between coincidental hypertension and PLMS and related arousals.[63]

Electrolyte concentrations

The present study suggests a potential relationship between sleep fragmentation and fluctuating electrolyte and vitamin D levels. A notable finding is the decreased Mg concentration observed in hypertensives as compared to normotensives. Additionally, there was an important decrease in vitamin D levels in hypertensives as compared to normotensives without OSA, although no significant difference was observed between hypertensives and normotensives.

Vitamin D metabolism

Vitamin D is primarily recognized for its role in maintaining Ca homeostasis and bone metabolism.[64] Animal studies indicated that vitamin D deficiency could lead to increased activity of RAAS and subsequent blood pressure elevation, suggesting a potential role for vitamin D as an antihypertensive agent. However, the results regarding the relationship between vitamin D and hypertension development remain contradictory.[65] A recent study involving 491 healthy middle-aged participants demonstrated a significant association between low vitamin D levels and non-optimal blood pressure, high-normal blood pressure and the development of hypertension over an 8-year follow-up period.[66] Additionally, physiological doses of vitamin D have been found to enhance Mg absorption.[67]

Magnesium and calcium metabolism

Mild to moderate hypomagnesemia has been associated with hypertension,[68] metabolic syndrome[69] and type 2 diabetes mellitus.[70,71] It is worth noting that Mg serves as a cofactor for 325 enzyme systems, including specific vasoactive mediators.[72] Intracellular Mg helps regulate vascular tone, while extracellular Mg influences Ca channels,[73] thereby impacting Ca metabolism. In the present study, Ca concentration was similar among the analyzed groups of patients. However, there was a correlation between Ca concentration and AI. Previous studies also showed a relationship between Ca signaling and the arousal state.[74] The thalamic T-type Ca channel is known to play a crucial role in blocking the transmission of arousal signals and promoting sleep stability.[75] Therefore, sleep fragmentation is associated with alterations in Ca metabolism and, indirectly, Mg and vitamin D metabolism in hypertensives with OSA.

Vitamin D deficiency is associated with increased OSA severity.[76,77] In this study, we observed a negative correlation between vitamin D concentration and AHI, as well as between vitamin D and the SpO_2 parameters, suggesting that hypoxemia may impact vitamin D concentration. However, it is important to note that there have been inconsistent findings regarding the relationship between vitamin D and sleep apnea, indicating the need for further studies to better understand this association.[78]

Uric acid metabolism

Studies consistently show that individuals with OSA tend to have higher levels of UA, which can be attributed to the hypothesis that the hypoxia associated with OSA leads to the breakdown of adenosine triphosphatase into xanthine, resulting in an elevated UA concentration.[79] According to a study conducted by Hira et al., the average level of serum UA was significantly higher in the group with OSA as compared to the control group and the standard laboratory reference value, and the association remained consistent after adjusting for factors such as sex, age and obesity.[80] These results align with previous research investigating UA levels in individuals with OSA.[81,82] It is important to note that serum UA level is also linked to hypertension, suggesting that UA may play a contributory and potentially causal role in primary hypertension.

The rise in the hypertension rates over the past century has been linked to increased sugar and salt consumption, and other components of the Western diet that can impact intracellular urate levels.[83] Hyperuricemia is a significant independent predictor of hypertension, with individuals experiencing a higher risk of developing hypertension within 5–10 years.[84,85] This study is consistent with previous research, as we observed significant differences in UA concentration between hypertensive patients with OSA and normotensive patients. However, further research is necessary to explore the underlying causes and mechanisms in greater detail.

In summary, individuals with hypertension have more severe OSA, an elevated frequency of PLMs, altered sleep architecture, sleep fragmentation, decreased sleep quality, a lower Mg concentration, and a higher UA concentration as compared to normotensive individuals.

Conclusions

Sleep architecture is altered in hypertensives with OSA. There is a reduction in the duration of REM sleep, an increase in WASO and a decrease in SE in comparison with normotensives.

Individuals with hypertension and OSA have an increased RAI, indicating sleep fragmentation. Calcium levels have been implicated in sleep fragmentation, suggesting a potential role for Ca and vitamin D in arousals. However, further studies are needed to investigate this relationship. Hypertension does not affect vitamin D concentration in patients with OSA, but a correlation between vitamin D and OSA severity has been established.

Uric acid and glucose concentrations are higher in hypertensives than in normotensives with OSA. Along with elevated AHI and sleep fragmentation, these factors contribute to an increased cardiovascular risk.

The periodic limb movement index is increased in hypertensives with OSA as compared to normotensives. However, PLMAI is similar in normotensives and hypertensives, suggesting no significant sleep fragmentation due to PLMs in hypertensives with OSA.

Magnesium concentration is decreased in hypertensives as compared to normotensives in OSA subjects. Therefore, hypertensives with OSA should be investigated for Ma insufficiency, and if present, supplementation should be considered.

Ethics approval and consent to participate

The study was approved by the Wroclaw Medical University Bioethical Committee (No. ID KB-407/2022) and adhered to the principles outlined in the Declaration of Helsinki. All patients provided written informed consent for the participation in the study.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Mechanism underlying the anti-diabetic potential of bee venom as compared to bone marrow mesenchymal stem cells in the diabetic rat tongue

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Abstract

Background. Diabetes mellitus (DM) is a critical chronic metabolic disease. Several treatment modalities are currently under investigation. Both bee venom (BV) and bone marrow mesenchymal stem cells (BMSCs) can possibly offer an approach for treating type I diabetes.

Objectives. The aim of the present study was to investigate the mechanism underlying the anti-diabetic effect of BV as compared to BMSCs on the tongue mucosa of diabetic rats.

Material and methods. A total of 52 male albino rats were used in the current study. The rats were randomly assigned into 4 groups: group 1 (control); group 2 (streptozocin (STZ)); group 3 (BV-treated); and group 4 (BMSC-treated). Diabetes mellitus was induced via an intraperitoneal (IP) injection of STZ in the rats from groups 2, 3 and 4. Following the diagnosis of DM, the rats in group 3 were injected with a daily dose of 0.5 mg/kg of BV, while the rats in group 4 were treated with a single injection of BMSCs. All rats were euthanized after 4 weeks, and their tongues were dissected and divided into halves. The right halves of the tongues were utilized for the histological examination, followed by morphometric analysis. In contrast, the left halves were used to detect the local gene expression of transforming growth factor beta 1 (TGF-β1) and vascular endothelial growth factor (VEGF).

Results. Group 2 revealed marked disruption in the morphology of the fungiform and filiform papillae, and atrophic epithelial changes in both dorsal and ventral surface epithelium as compared to other groups. Group 4 showed a significantly larger number of taste buds, and a higher gene expression of *TGF-β1* and *VEGF* as compared to groups 2 and 3. Additionally, BV and BMSCs effectively increased the thickness of dorsal and ventral surface epithelium as compared to group 2.

Conclusions. Treatment with BMSCs was associated with significant improvement in the morphology and number of lingual epithelial cells and taste buds in the tongues of diabetic rats as compared to BV-treated rats, which was due to the local upregulation of *TGF-β1* and *VEGF* gene expression.

Keywords: bee venom, diabetes, streptozocin, bone marrow mesenchymal stem cells

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Introduction

Diabetes mellitus (DM) is considered a critical chronic metabolic disease due to a prompt rise in the number of diagnosed patients, especially over the last 2 decades.[1] Diabetes mellitus is characterized by high blood glucose levels, which, if not controlled efficiently, leads to end-organ damage within the genitourinary, cardiovascular and neurological systems.[2] These characteristics make DM a condition that needs proficient and long-standing medical care in order to control elevated blood glucose levels and prevent complications.[3] Several drugs used to treat DM have adverse effects, which necessitates a search for other modalities that could overcome these side effects, without imposing a financial strain on the patient.[4]

Bee venom (BV) is composed of different types of proteins, enzymes and non-peptide components. The proteins include melittin, apamin, adolapin, and mast cell degranulating (MCD) peptide. The enzymes found in BV include α-glucosidase, phosphatase, phospholipase B, phospholipase A2, and hyaluronidase. Additionally, non-peptide components, such as histamine, dopamine and norepinephrine, have been identified in BV.[5] Due to its therapeutic effects, BV has been used as a drug in the treatment of many diseases, such as cardiovascular, neurological, hematological, musculoskeletal, and dermatological diseases.[6,7]

The anti-diabetic effect of BV can be associated with melittin and phospholipase A2, a polypeptide and an enzyme that increase the secretion of insulin from pancreatic β-cells via the depolarization of the β-cell membrane.[8] Taking into account the abovementioned properties, BV could be considered a therapeutic agent in the treatment of DM.

Bone marrow mesenchymal stem cells (BMSCs) are multipotent, self-renewing cell populations that exhibit a marked therapeutic potential because of their ability to differentiate into the 3 germ layer lineages. Furthermore, they can migrate toward the damaged sites when administered systemically. In damaged tissues, they can improve recovery, as they differentiate into cells specific to the tissue, and produce paracrine mediators and trophic factors that possess anti-apoptotic properties and stimulate cell proliferation.[9–11]

The BMSC therapy offers a cell-based approach for treating type I diabetes. These cells can differentiate into insulin-producing cells, and also show immunosuppressive activity, exerting ameliorative effects on injured tissues.[12,13]

Diabetes mellitus is often related to prolonged or insufficient healing; this is attributed to the abnormal inflammatory response and a reduction in the production of growth factors, leading to impaired neovascularization and compressed collagen matrices.[14]

Transforming growth factor beta 1 (TGF-β1) is known to regulate the chemotaxis of immune and inflammatory cells, cellular differentiation, and induce the accumulation of extracellular matrix proteins.[15] Indeed, defective TGF-β1 signaling contributes to delayed wound healing in diabetes.[16] Vascular endothelial growth factor (VEGF) promotes tissue repair via increasing vascular permeability, as well as the proliferation and migration of the pre-existing endothelial cells.[17]

The present study aimed to evaluate and compare the potential anti-diabetic effects of BV and BMSCs against the histological and molecular changes in the tongue in streptozotocin (STZ)-induced diabetic albino rats.

Material and methods

Bee venom

The BV samples were collected from the colonies of Italian and Carniolan hybrid honeybees (*Apis mellifera*), using a BV collector (an electric shock device, VC-Starter kit; IGK electronics Ltd., Varna, Bulgaria) at the National Research Center, Cairo, Egypt. The dried BV material was transferred to a proper container and was solubilized in distilled water to reach a concentration of about 0.1 mg/mL.[18]

Bone marrow mesenchymal stem cells (BMSCs)

Bone marrow mesenchymal stem cells were isolated from the femora and tibiae of 6 Wistar donor rats (6-week-old, male, weighing 100 ±20 g). The isolation and propagation of BMSCs was conducted under aseptic conditions, 14 days before the experimental procedures, as previously described.[19]

The femora and tibiae were flushed with Dulbecco's Modified Eagle Medium (DMEM) (Gibco-BRL, Gaithersburg, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco-BRL), using an 18-gauge needle. The cells were cultured in a culture medium supplemented with 1% penicillin–streptomycin (Gibco-BRL) in an incubator, at 37°C in a humidified atmosphere (5% CO_2). Upon reaching 80–90% confluence, the cultures were washed twice with phosphate-buffered saline (PBS) and the cells were trypsinized with 0.25% trypsin in 1 mM ethylenediaminetetraacetic acid (EDTA) (Gibco-BRL) at 37°C for 5 min. Then, the cells were centrifuged at 2,400 rpm for 20 min, re-suspended with a serum-supplemented medium and incubated in a Falcon® culture flask with a surface area of 50 cm^2. On day 14, the adherent colonies of cells were trypsinized and counted.[19]

Culture confluence was monitored using an inverted light microscope (Olympus, Center Valley, PA, USA) with a digital camera (Nikon, Tokyo, Japan).

Characterization of BMSCs

Flow cytometry was performed to identify BMSCs. After blocking in 0.5% bovine serum albumin (BSA) and 2% FBS in PBS, 100,000 cells were incubated in the dark at 4°C for 20 min with the following monoclonal antibodies: FITC CD 90 (PN IM1839U; Beckman Coulter, Brea, USA); and PE CD 34 (PN IM1871U; Beckman Coulter). Mouse-isotype PE antibodies (Beckman Coulter) were used as controls (the dilution of all antibodies at 1:1,500). The cells were washed and suspended in 500 µL of the fluorescence-activated cell sorting (FACS) buffer, and analyzed using the Cytomics FC 500 flow cytometer with the CPX software, v. 2.2 (Beckman Coulter).

Animals

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at Cairo University, Egypt (approval No. CU III F 74 18). This research was conducted in compliance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and regulations (https://arriveguidelines.org).

A sample size of 52 (13 per group) was estimated to be sufficient, assuming an effect size of 0.6, a power of 0.8, a two-sided hypothesis test, and a significance level of 0.05 for categorical and numerical data. Fifty-two healthy adult male albino rats, weighing about 150–200 g, with normal glucose levels, were used. All animals were housed in a sterile, controlled environment (room temperature of 25 ±5°C under a natural light, 12-hour dark/light cycle). They were fed with standard, ordinary, commercial rat pellets and were provided water ad libitum. The maintenance and care of the experimental animals conformed with the International Guiding Principles for Biomedical Research Involving Animals. The animals were randomly divided into 4 groups of 13 animals, using the Random Sequence Generator program (https://www.random.org).

Induction of experimental diabetes

Streptozotocin (Sigma-Aldrich, St. Louis, USA)[20] was used for inducing type I diabetes by a single intraperitoneal (IP) injection of STZ (60 mg/kg b.w.) into fasted rats. The rats were considered diabetic when their random blood glucose reading was >300 mg/dL at 72 h of the STZ injection.[21] Diabetes was allowed to stabilize in the STZ-injected rats for 1 week. The assessment of blood glucose levels was performed weekly in all diabetic rats until the end of the experiment. In all groups, the blood samples were obtained from the tail vein and the blood glucose level was expressed in mg/dL.

Animal grouping

The animals were randomly divided into 4 groups, 13 animals in each, occupying separate cages (Table 1). Group 1 (control) comprised normal healthy rats, which were given a physiological saline solution (0.9% NaCl). The veterinarian administered the STZ induction. Then, the STZ-treated rats were randomly divided into 3 groups (2–4). The rats from group 2 (the STZ group) received a single IP injection of STZ (60 mg/kg in freshly prepared 0.1 mol/L citrate buffer (pH 4.5)) and were left untreated. The diabetic rats from group 3 (the BV-treated diabetic group) were injected IP with a daily dose of 0.5 mg/kg of BV for 4 weeks.[22] The diabetic rats from group 4 (BMSC-treated diabetic group) were administered a single intravenous (IV) injection of the previously cultured BMSCs at a dose of 1 million cells/mL in PBS.[23]

Animal sacrifice and tissue preparation

All rats were euthanized by an intracardiac overdose of sodium thiopental (80 mg/kg) after 4 weeks. The tongues were dissected into 3 parts: two halves of the anterior two-thirds; and the posterior one-third of the tongue.

The specimens from the right halves of the anterior two-thirds and the posterior one-third of the tongue were prepared for the histopathological examination. Serial sections of 5 µm of the tongue tissues were cut and subjected to the following:

– the fluorescence detection of the PKH26-labeled BMSCs in the unstained paraffin sections with the use of a fluorescence microscope; and
– the hematoxylin and eosin (H&E) staining for histological evaluation.

The specimens from the left halves of the anterior two-thirds were used to measure the expression of both TGF-β1 and VEGF.

The assessment steps were carried out blindly by the investigators.

Table 1. Experimental design of the study

Group	*n*	Induction of diabetes	Treatment
Group 1 (control)	13 rats	none	saline solution
Group 2 (STZ)	13 rats	IP injection of STZ	none
Group 3 (STZ + BV)	13 rats	IP injection of STZ	injected IP with a daily dose of 0.5 mg/kg of BV for 4 weeks
Group 4 (STZ + BMSCs)	13 rats	IP injection of STZ	a single IV injection of the previously cultured BMSCs at a dose of 1 million cells/mL in PBS

STZ – streptozotocin; BV – bee venom; BMSCs – bone marrow mesenchymal stem cells; IP – intraperitoneal; IV – intravenous; PBS – phosphate-buffered saline.

Morphometric analysis

The specimens were examined using light microscopy (Leica, St. Gallen, Switzerland) under ×400 magnification. The data was obtained using the Leica Qwin 500 image analyzer computer system (Leica Biosystems, Cambridge, UK). Image analysis was done using the ImageJ software, v. 1.53d (https://imagej.net/ij).[24] The image analysis system was used to assess the dorsal and ventral epithelial thickness, the thickness of each cell layer,[25] and the area of the connective tissue papillae.[26] For each criterion, 5 non-overlapping microscopic fields were randomly selected and evaluated. As previously described, the number of taste buds per circumvallate papilla was assessed.[27]

Quantitative real-time polymerase chain reaction

To analyze the mRNA levels of TGF-β1 and VEGF, the total RNA-containing genes were determined by the quantitative real-time polymerase chain reaction (qRT-PCR). RNA was isolated with the QIAzol lysis reagent (Qiagen, Venlo, the Netherlands). Complementary DNA (cDNA) was produced using a cDNA synthesis kit (Applied Biosystems, Waltham, USA), and qRT-PCR was performed using the StepOnePlus™ RT-PCR system (Applied Biosystems), following the standardized protocols. The expression of genes was normalized relative to the mean critical threshold (CT) values with the ΔΔCT method, using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA as an internal control. The primers for *TGF-β1*, *VEGF* and *GADPH* are listed in Table 2.

Statistical analysis

The values are presented as mean and standard deviation ($M \pm SD$). The Kolmogorov–Smirnov test indicated that the data was normally distributed. The one-way analysis of variance (ANOVA) was used to assess differences between the groups, and Tukey's post hoc test was applied when ANOVA yielded a significant difference. The significance level was set at $p < 0.05$. Statistical analysis was performed using the Minitab® software, v. 18.1 (https://www.minitab.com/en-us/support/downloads).

Table 2. Primer sequences specific for each gene

Gene	Primer sequence from 5' to 3'
TGF-β1	forward: TAC CAT GCC AAC TTC TGT CTG GG A reverse: ATG TTG GAC AAC TGC TCC ACC TTG
VEGF	forward: CAC CAC CAC ACC ACC ATC reverse: GCG AAT CCA GTT CCA CGAG
GAPDH	forward: ACA GTC CAT GCC ATC ACT GCC reverse: GCC TGC TTC ACC ACC TTC TTG

Results

Characteristics of BMSCs in the culture

The cultured BMSCs revealed a fibroblast-like morphology and they adhered to the tissue culture substrate within 24–48 h. They reached confluence within 7–14 days (Fig. 1).

Fluorescence detection

The examination of the unstained paraffin sections with the use of a fluorescence microscope was performed to detect and track the PKH26-labeled BMSCs. The tongue specimens from group 4 injected with the PKH26-labeled BMSCs showed red fluorescent cells within the tongue tissue (Fig. 2).

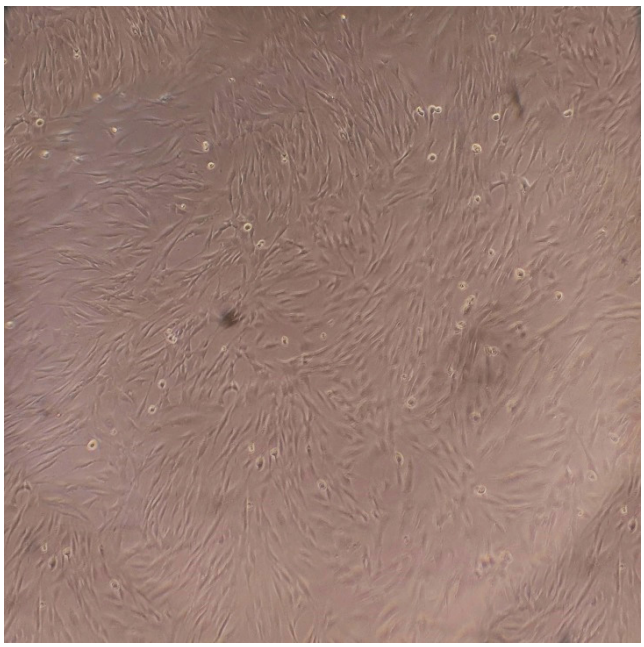

Fig. 1. Microscopic observation shows that the isolated bone marrow mesenchymal stem cells (BMSCs) have a fibroblast-like and spindle-shaped morphology (×100)

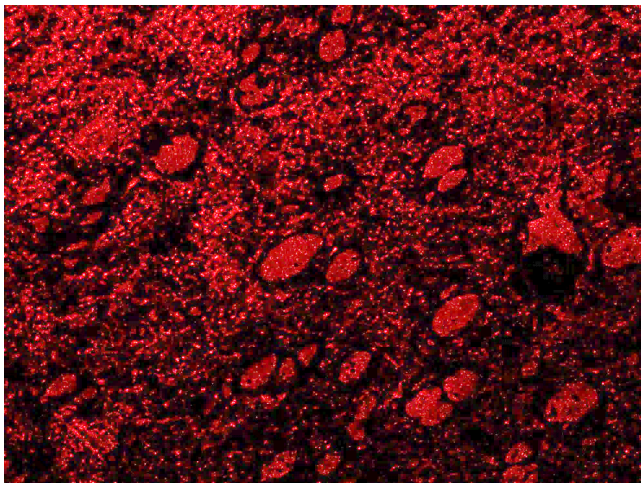

Fig. 2. Photomicrograph of group 4 tongue specimens, showing the PKH26-labeled bone marrow mesenchymal stem cells (BMSCs), which appear as red fluorescent cells

Characterization of BMSCs

The cells were characterized by flow cytometry through the use of surface markers. The results showed that most of the BMSCs were positive for CD90 and negative for CD34 (Fig. 3).

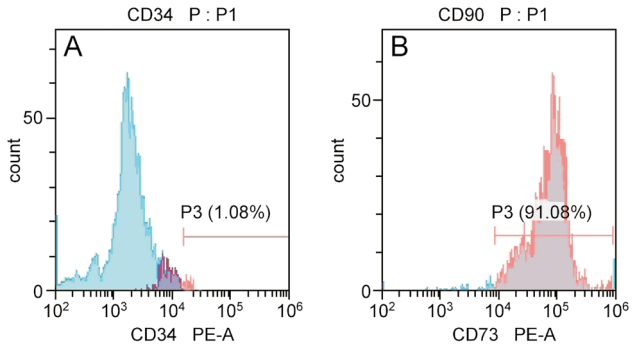

Fig. 3. Flow cytometric analysis showing the expression level of $CD90^+$ (A) and $CD34^-$ (B) in the bone marrow mesenchymal stem cells (BMSCs) isolated from the rats

Biochemical analysis

At 3 weeks, BMSCs significantly reduced blood glucose levels as compared to the STZ group. At 4 weeks, both BV and BMSC treatment were associated with a significant reduction in blood glucose levels as compared to the STZ group (Table 3).

Histopathological examination

Upon the examination of group 1 (the control group), the fungiform papillae displayed a normal mushroom-like appearance with barrel-shaped taste buds and well-defined connective tissue (Fig. 4A). Normal thread-like filiform papillae with orthokeratinized epithelium and normal underlying connective tissue were detectable (Fig. 5A). The circumvallate papillae showed normal barrel-shaped taste buds (Fig. 6A). Dorsal and ventral surface epithelium revealed a normal thickness, was covered with normal orthokeratin and showed normal underlying connective tissue (Fig. 7A and 8A).

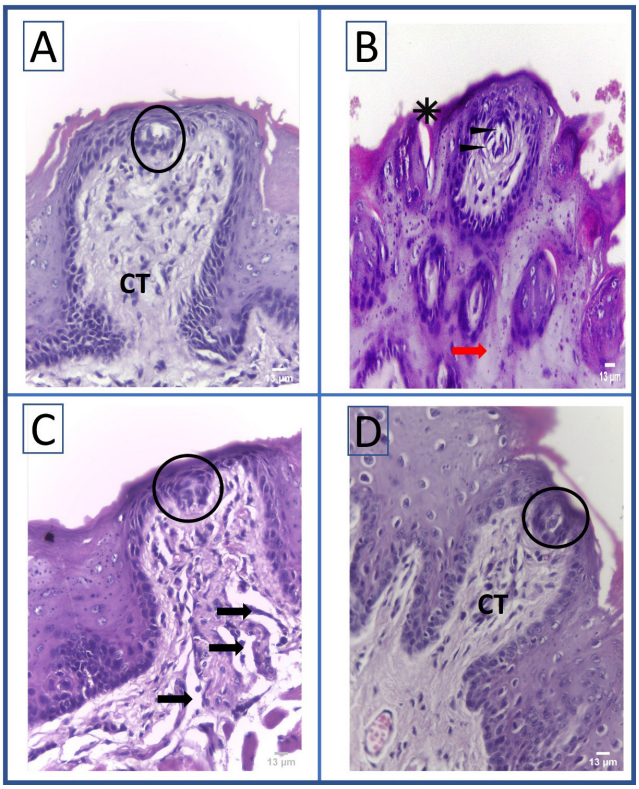

Fig. 4. Photomicrograph of the fungiform papillae

A – group 1 (control) showing normal mushroom-like papillae with well-defined connective tissue (CT) and barrel-shaped taste buds (circle); B – group 2 (STZ) showing disruption in the papillae, a detached keratin layer (asterisk), connective tissue degeneration (red arrow), and taste buds with intracellular vacuolation (black arrow heads); C – group 3 (STZ + BV) showing mushroom-like papilla and barrel-shaped taste buds (circle) besides areas of connective tissue degeneration (black arrows); D – group 4 (STZ + BMSCs) showing mushroom-like papillae with well-defined connective tissue (CT) and normal barrel-shaped taste buds (circle). Original magnification ×400, scale bar: 13 µm.

Group 2 (the STZ group) showed disruption in the normal mushroom-like appearance of the fungiform papillae, and areas of a detached keratin layer and connective tissue degeneration. The fungiform papilla-associated taste buds revealed intracellular vacuolation and evidence of degeneration (Fig. 4B). The filiform papillae displayed atrophied, rounded tips, and a thin layer of keratin with areas of detached keratin. Some epithelial cells revealed hyperchromatic nuclei. The underlying connective tissue displayed evidence

Table 3. Descriptive statistics and comparison of blood glucose levels [mg/dL] between the studied groups (ANOVA and Tukey's post hoc test)

Time point	Group 1	Group 2	Group 3	Group 4	*p*-value
Week 1	118.05 ±14.35^B	334.14 ±22.19^A	325.25 ±13.93^A	316.5 ±54.2^A	0.000*
Week 2	114.14 ±14.90^B	338.14 ±21.09^A	327.75 ±13.97^A	322.9 ±49.1^A	0.000*
Week 3	113.47 ±14.26^C	355.61 ±18.94^A	331.10 ±14.85^{A,B}	325.7 ±43.2^B	0.000*
Week 4	112.81 ±14.37^C	361.91 ±15.85^A	334.54 ±14.84^B	323.8 ±39.0^B	0.000*

Data presented as mean ± standard deviation (*M* ±*SD*).
Group 1 – control; group 2 – STZ; group 3 – BV-treated diabetic rats; and group 4 – BMSC-treated diabetic rats. * statistically significant; the values with different superscript letters are significantly different.

of degeneration (Fig. 5B). The circumvallate papillae showed multiple degenerated taste buds, while some taste buds presented the loss of their normal barrel-shaped outline (Fig. 6B). Dorsal surface epithelium revealed a reduced thickness and areas of detached keratin. In a few epithelial cells, hyperchromatic nuclei were detectable. There were areas of connective tissue degeneration and dilated blood vessels (Fig. 7B). The epithelium covering the ventral surface of the tongue showed epithelial atrophy, while the connective tissue showed areas of degeneration and dilated blood vessels (Fig. 8B).

Group 3, treated with BV, showed normal fungiform papillae with a mushroom-like appearance and normal barrel-shaped taste buds, with areas of connective tissue vacuolation (Fig. 4C). The examination of the filiform papillae revealed a thread-like shape, and multiple atrophied, rounded tips with areas of detached keratin (Fig. 5C). The circumvallate papillae showed few degenerated taste buds (Fig. 6C). Dorsal and ventral surface epithelium showed increased epithelial and keratin thickness as compared to Group 2, and areas of connective tissue degeneration (Fig. 7C and 8C).

The examination of group 4, treated with BMSCs, showed mushroom-like fungiform papillae, with normal barrel-shaped taste buds and well-defined connective tissue (Fig. 4D). Thread-like shaped filiform papillae with few areas of detached keratin were detectable (Fig. 5D). Normal circumvallate papillae with normal barrel-shaped taste buds were evident (Fig. 6D). Dorsal and ventral surface epithelium showed an increase in the epithelium and keratin thickness as compared to groups 2 and 3, and areas of connective tissue degeneration (Fig. 7D and 8D).

Morphometric analysis

The highest number of taste buds within the circumvallate papillae was detected in group 1, while the lowest was recorded in group 2, with a statistically significant difference between the groups ($p < 0.05$). Pairwise comparisons revealed a significantly higher number of taste buds in group 4 as compared to groups 2 and 3 ($p < 0.05$). Also, a significantly higher number was recorded in group 3 as compared to group 2 ($p = 0.002$) (Table 4).

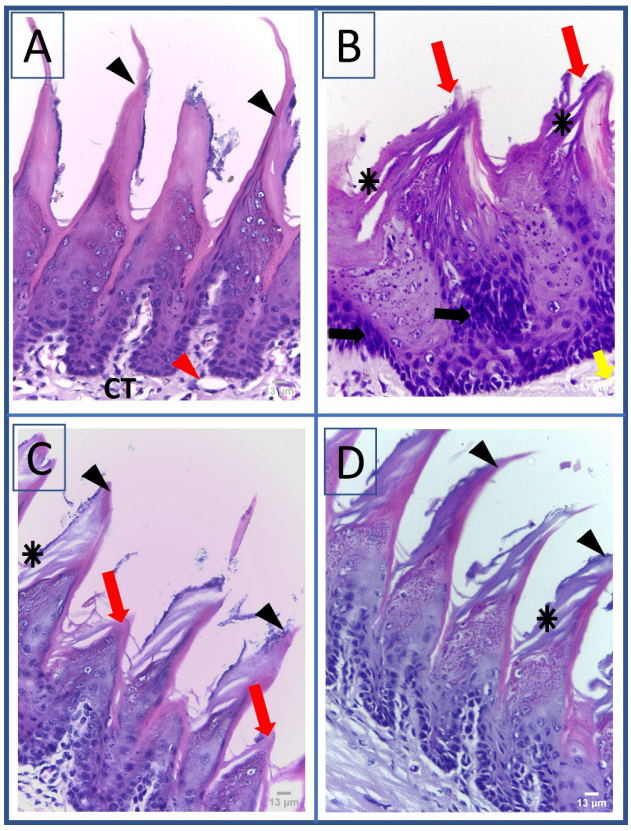

Fig. 5. Photomicrograph of the filiform papillae

A – group 1 (control) showing normal thread-like papillae with orthokeratinized epithelium (black arrow heads), and normal underlying connective tissue (CT) and blood vessels (red arrow head); B – group 2 (STZ) showing the papillae with atrophied rounded tips (red arrows), a detached keratin layer (asterisk), connective tissue degeneration (yellow arrow), and some hyperchromatic nuclei (black arrows); C – group 3 (STZ + BV) showing a thread-like shape (black arrow heads), atrophied, rounded tips (red arrows) and areas of detached keratin (asterisk); D – group 4 (STZ + BMSCs) showing thread-like papillae (black arrow heads) and areas of detached keratin (asterisk). Original magnification ×400, scale bar: 13 µm.

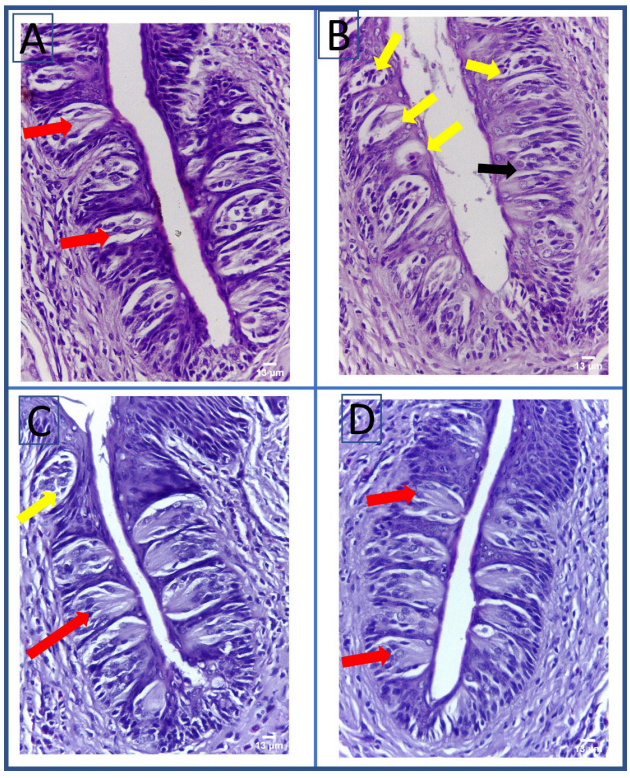

Fig. 6. Photomicrograph of the circumvallate papillae surrounded by a trough with taste buds

A – group 1 (control) showing normal barrel-shaped taste buds (red arrows); B – group 2 (STZ) showing multiple degenerated taste buds (yellow arrows) and taste buds with the loss of their normal barrel-shaped outline (black arrow); C – group 3 (STZ + BV) showing normal barrel-shaped taste buds (red arrow) and few degenerated taste buds (yellow arrow); D – group 4 (STZ + BMSCs) showing normal barrel-shaped taste buds (red arrows). Original magnification ×400, scale bar: 13 µm.

Group 2 was associated with atrophic epithelial changes, with a significantly reduced thickness of both dorsal and ventral surface epithelium as compared to other groups ($p < 0.05$). Groups 3 and 4 effectively increased the epithelial thickness, which showed significantly higher values in both dorsal and ventral surface epithelium as compared to group 2 ($p < 0.05$). Regarding the dorsal and ventral epithelial thickness, a higher mean value was reported for group 4 as compared to group 3. However, the difference was not statistically significant (Table 4).

A reduction in the dorsal epithelial thickness in group 2 as compared to other groups was associated with a significant reduction in the thickness of the basal cell, polyhedral cell, granular cell, and keratin layers ($p < 0.05$) (Table 4).

Both group 3 and group 4 showed a significant increase in the thickness of polyhedral cell, granular cell and keratin layers as compared to group 2 ($p < 0.05$), whereas the thickness of the basal cell layer showed a significant increase only in group 4 as compared to group 2 ($p < 0.05$). A higher mean value was recorded for the epithelial layer thickness in group 4 as compared to group 3; however, the difference was not statistically significant except for the polyhedral cell layer thickness ($p < 0.05$) (Table 4).

Group 2 showed a significant reduction in the area of the connective tissue papillae ($p < 0.05$). Group 3 had significantly increased connective tissue papillae as compared to groups 2 and 4 ($p < 0.05$). An insignificantly higher mean area of the connective tissue papillae was detected in group 4 as compared to group 2 ($p < 0.05$) (Table 4).

TGF-β1 and *VEGF* gene expression

Group 2 showed a significant reduction in *TGF-β1* and *VEGF* expression as compared to other groups ($p < 0.05$). Group 4 showed a significant increase in *TGF-β1* and *VEGF* expression as compared to groups 2 and 3 ($p < 0.05$) (Table 5).

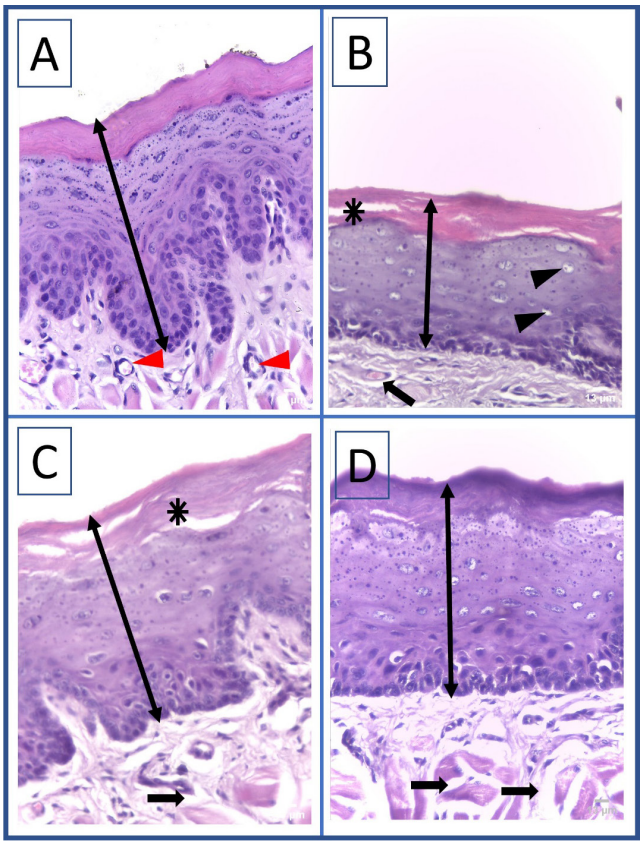

Fig. 7. Photomicrograph of the epithelium covering the dorsal surface of the tongue

A – group 1 (control) showing a normal epithelial thickness (black double arrow), normal orthokeratinizied epithelium and normal blood vessels (red arrow heads); B – group 2 (STZ) showing a reduced epithelial thickness (black double arrow), areas of detached keratin (asterisk), intra-epithelial vacuolation (black arrow heads), areas of connective tissue degeneration (black arrow), and dilated blood vessels; C – group 3 (STZ + BV) showing an increased epithelial thickness (black double arrow), areas of detached keratin (asterisk) and areas of connective tissue degeneration (black arrow); D – group 4 (STZ + BMSCs) showing an increase in the epithelium thickness (black double arrow) and areas of connective tissue degeneration (black arrows). Original magnification ×400, scale bar: 13 µm.

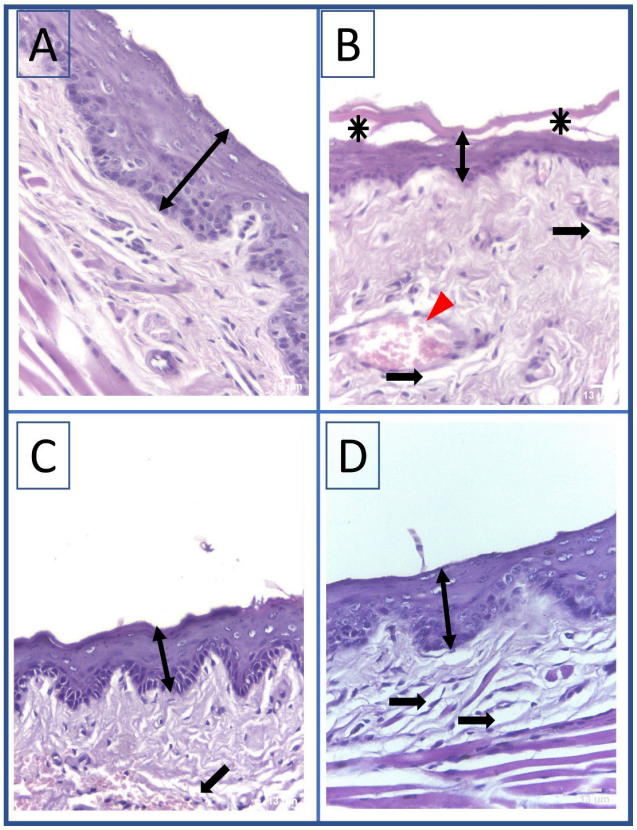

Fig. 8. Photomicrograph of the epithelium covering the ventral surface of the tongue

A – group 1 (control) showing a normal epithelial thickness (black double arrow); B – group 2 (STZ) showing a reduced epithelial thickness (black double arrow), areas of detached keratin (asterisks), areas of connective tissue degeneration (black arrows), and dilated blood vessels (red arrow head); C – group 3 (STZ + BV) showing an increased epithelial thickness (black double arrow) and areas of connective tissue degeneration (black arrow); D – group 4 (STZ + BMSCs) showing an increase in the epithelium thickness (black double arrow) and areas of connective tissue degeneration (black arrows). Original magnification ×400, scale bar: 13 µm.

Table 4. Morphometric analysis of the studied groups (ANOVA and Tukey's post hoc test)

Parameter	Group	*M* ±*SD*	*SE*	95% *CI*		F	*p*-value (ANOVA)
				lower bound	upper bound		
Number of taste buds per circumvallate papilla	group 1^{A}	18.214 ±1.323	0.367	17.179	19.249	98.04	0.000*
	group 2^{D}	6.308 ±1.601	0.444	5.272	7.343		
	group 3^{C}	9.154 ±2.968	0.823	8.118	10.189		
	group 4^{B}	12.183 ±0.817	0.227	11.147	13.218		
Dorsal epithelial thickness [mm]	group 1^{A}	237.03 ±25.53	7.08	222.53	251.53	28.77	0.000*
	group 2^{C}	145.62 ±9.36	2.60	131.12	160.12		
	group 3^{B}	195.44 ±7.63	2.11	180.94	209.94		
	group 4^{A,B}	212.70 ±43.70	12.10	198.20	227.20		
Ventral epithelial thickness [mm]	group 1^{A}	141.64 ±27.30	7.57	127.66	155.61	28.10	0.000*
	group 2^{C}	54.65 ±5.02	1.39	40.67	68.62		
	group 3^{B}	100.60 ±38.50	10.70	86.60	114.50		
	group 4^{A,B}	118.41 ±16.06	4.45	104.44	132.38		
Basal cell layer thickness [mm]	group 1^{A}	12.725 ±1.693	0.470	11.728	13.722	15.89	0.000*
	group 2^{C}	8.265 ±2.114	0.586	7.268	9.262		
	group 3^{B,C}	9.007 ±1.766	0.490	8.010	10.004		
	group 4^{B}	10.621 ±1.525	0.423	9.624	11.618		
Polyhedral cell layer thickness [mm]	group 1^{A}	55.87 ±14.25	3.95	46.49	65.24	50.19	0.000*
	group 2^{C}	130.28 ±12.00	3.33	120.90	139.65		
	group 3^{B}	115.21 ±20.51	5.69	105.83	124.50		
	group 4^{A}	84.96 ±19.06	5.29	75.58	94.33		
Granular cell layer thickness [mm]	group 1^{A}	62.49 ±18.92	5.25	55.65	69.33	17.70	0.000*
	group 2^{C}	29.04 ±3.94	1.09	22.21	35.88		
	group 3^{B,A}	40.50 ±9.67	2.68	33.66	47.34		
	group 4^{A,B}	51.01 ±11.60	3.22	44.17	57.84		
Keratin layer thickness [mm]	group 1^{A}	44.14 ±10.81	3.00	−19.54	0.55	16.41	0.000*
	group 2^{B}	20.82 ±1.47	5.29	15.68	25.96		
	group 3^{A}	37.64 ±12.55	3.48	−13.43	6.66		
	group 4^{A}	40.76 ±6.14	1.70	−7.05	13.05		
Area of the connective tissue papillae [mm^2]	group 1^{A}	2,298.0 ±1,648.0	61.8	1,776.9	2,725.4	15.49	0.000*
	group 2^{B}	636.6 ±176.2	48.9	162.4	1,110.9		
	group 3^{A}	2,251.2 ±222.9	457.0	1,824.0	2,773.0		
	group 4^{B}	698.7 ±311.9	86.5	224.5	1,173.0		

SE – standard error; *CI* – confidence interval; * statistically significant; different superscript letters indicate significant differences between the groups.

Table 5. Descriptive statistics and comparison of *TGF-β1* and *VEGF* gene expression between the studied groups (ANOVA and Tukey's post hoc test)

Parameter	Group	*M* ±*SD*	*SE*	95% *CI*		F	*p*-value (ANOVA)
				lower bound	upper bound		
TGF-β1	group 1^{A}	5.6631 ±0.1126	0.0312	5.6092	5.7170	98.04	0.000*
	group 2^{D}	1.0550 ±0.0657	0.0182	1.0011	1.1089		
	group 3^{C}	2.2992 ±0.0994	0.0276	2.2453	2.3531		
	group 4^{B}	2.8300 ±0.1023	0.0284	2.7761	2.8839		
VEGF	group 1^{A}	4.1785 ±0.0584	0.0162	4.1386	4.2183	28.77	0.000*
	group 2^{D}	1.0750 ±0.0704	0.0195	1.0352	1.1148		
	group 3^{C}	1.6669 ±0.0821	0.0228	1.6271	1.7068		
	group 4^{B}	2.0873 ±0.0728	0.0202	2.0475	2.1271		

* statistically significant; different superscript letters indicate significant differences between the groups.

Discussion

Diabetes mellitus is a metabolic disorder resulting in multiple complications, including soft tissue abnormalities in the oral cavity. In the present study, we investigated the possible therapeutic effects of BV and BMSCs on the degenerative changes affecting the tongue due to the induction of DM in rats.

Streptozotocin was chosen for DM induction in rats, as it provides a permanent and stable diabetic model. It can induce type I diabetes through the rapid destruction of β-cells in the pancreas.[28] In the current study, STZ-induced DM in rats was associated with atrophic changes to the lingual mucosa, with a reduction in the thickness of both dorsal and ventral surface epithelium, the atrophy of the lingual papillae, and a reduction in the number of taste buds per circumvallate papilla. The atrophy of the underlying connective tissue with a significant reduction in the connective tissue papillae was also noticed.

The effect of uncontrolled DM on rats' tongue epithelium and papillae has been previously reported. Streptozotocin-induced DM in rats has been associated with alterations in the distribution and morphology of the fungiform and filiform papillae, with areas of epithelial desquamation,[29–31] in addition to a significant reduction in the height and width of the filiform papillae.[32] Diabetes mellitus has also been associated with the atrophy and a decreased thickness of dorsal surface epithelium,[32–34] and the atrophy of ventral surface epithelium.[34] Lingual epithelial atrophy was observed in rats born to diabetic mothers.[35] Epithelial atrophy was also noticeable in the buccal mucosa of diabetic rats.[36]

In the present study, the circumvallate papillae were impacted by DM. A significant reduction in the number of taste buds per circumvallate papilla has been reported in previous studies.[27,37] These changes were attributed to a DM-associated reduction in the innervation of taste buds.[27] Additionally, rats with induced DM were prone to increased apoptosis of circumvallate papilla taste buds, which was associated with the downregulation of Bcl-2, the upregulation of Bax, and increased activation of caspase-9 and caspase-3.[37]

Diabetes mellitus has also been linked to the atrophy of the lamina propria underlying the lingual mucosa. A decrease in the height and cross-sectional area of the connective tissue papillae of the dorsal surface of the tongue in rats with induced DM has been previously reported.[26,31] In addition to the atrophied gingival lamina propria,[25,38] DM was also linked to an increased incidence of *Candida albicans* and the thickening of bacterial colonies on the dorsal surface of the tongue.[29]

On the other hand, some reports demonstrated that DM was associated with epithelial hypertrophy.[25,38] The thickness of the gingival epithelium, and of the prickle cell, granular cell and keratin layers was significantly higher in rats with induced DM as compared to the control group.[25] A statistically significant increase in the thickness of the gingival epithelium, and of the basal, prickle cell, granular, and keratin layers was also observed.[38] This indicates that regional variations in the oral tissues can affect the mucosal response to chemically induced DM in rats.

The atrophic effect of DM on the lingual mucosa can be traced back to the diabetes effect on epithelial cells, as DM has been associated with reduced keratinocyte proliferation[39] and increased cellular apoptosis.[40] Reduced cell proliferation, in addition to increased cellular apoptosis, can result in epithelial atrophy. The effect of DM on cellular functions has been attributed to high blood glucose levels, the accumulation of advanced glycation end-products (AGEs), increased tissue hypoxia, and increased levels of reactive oxygen species (ROS) in DM.[41–43] Diabetes mellitus can also cause systemic inflammation and a chronic inflammatory infiltrate in the oral connective tissues, which can negatively affect the integrity and function of the oral mucosa.[44–47] Additionally, diabetic microangiopathy may lead to subsequent connective tissue degeneration and mucosal atrophy.[25,26,48] It has been observed that DM is also linked to an increased expression of p53 in rats[33] and an increased expression of p16.[32]

The positive effect of BV on the lingual mucosa observed in the current study reflects its potent biological properties. Bee venom shows anti-inflammatory,[49] antibacterial[50] and antioxidant effects.[51] In addition, BV has demonstrated anti-cancerous effects[52,53] and anti-obesity effects.[53] It also has an anti-diabetic effect, as it can lower a blood glucose level, increase the secretion of insulin[53] and enhance diabetic wound healing.[51,53–56] The anti-inflammatory activity of BV could be attributed to melittin, its main constituent, as melittin inhibits the enzymatic activity of phospholipase A2.[51] The phospholipase A2 enzyme is released in severe inflammatory disorders and causes tissue damage.

Bone marrow mesenchymal stem cells effectively improved the morphology of the lingual papillae, increased the number of taste buds per circumvallate papilla and effectively reversed DM-associated epithelial atrophy. Similar to our findings, BMSCs effectively reversed atrophic changes in the fungiform and filiform papillae,[30,34,57] lingual mucosa,[34] and increased the ventral epithelial thickness in rats with STZ-induced DM.[34] Bone marrow mesenchymal stem cells were also reported to improve the morphology of both the circumvallate and foliate papillae, and restore their taste buds in rats with STZ-induced DM.[58] The regenerative effect of BMSC on the lingual mucosa can be attributed to the ability of the cells to release different signaling molecules, including growth factors, cytokines and chemokines, in addition to their anti-inflammatory effect.[59]

The ability of BMSCs to restore the lingual mucosa in diabetic rats can be partly attributed to their angiogenic effect. Bone marrow mesenchymal stem cells can induce

angiogenesis in diabetic wounds through the release of pro-angiogenic factors, including hypoxia-inducible factor (HIF), VEGF, angiopoietin, and erythropoietin,[60,61] in addition to their ability to differentiate into endothelial cells.[61] They can also secrete multiple growth factors, including epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1) and platelet-derived growth factor (PDGF), important for the chemotaxis and function of cells responsible for diabetic wound healing.[62,63] The cells also have immunomodulatory properties. They can interact with cells of the innate and adaptive immune systems to downregulate pro-inflammatory cytokines, including interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α) and IL-6, and to upregulate anti-inflammatory cytokines – IL-10 and prostaglandin E2 (PGE2).[64] Bone marrow mesenchymal stem cells can also increase the migration, proliferation and function of keratinocytes in diabetic wounds, and induce re-epithelialization.[63,65] Taken together, the ability of BMSCs to stimulate angiogenesis and keratinization, and their immunomodulatory properties can explain their potential to reverse the diabetes-associated atrophic changes observed in the lingual mucosa of rats in the current study.

In this study, the levels of TGF-β1 and VEGF were investigated to define their role in enhancing the regeneration of the tongue following BV and BMSC treatment in diabetic rats. Treatment with BV and BMSCs resulted in the upregulation of both TGF-β1 and VEGF in comparison with the levels observed in the diabetic rats in group 2.

The molecular mechanisms underlying the regeneration of damaged tongue tissues following BV treatment in group 3 may be related to the ability of BV to upregulate TGF-β1, which stimulates the migration of keratinocytes and increases integrin expression.[66] Transforming growth factor beta 1 can induce collagen expression.[67] Also, BV enhances the expression of VEGF, which in turn stimulates local angiogenesis through mobilizing and recruiting bone marrow-derived endothelial progenitor cells, thereby decreasing impaired healing in diabetic rats.[51]

According to Kwon et al., the regenerative potential of BMSCs in tongue tissues in diabetic rats appeared to provide better results when compared to the untreated animals, which was explained by the local upregulation of cytokines and growth factors.[62] Bone marrow mesenchymal stem cells increase the expression of TGF-β1 moderately and of VEGF markedly, which in turn enhances the inflammatory response, and induces the recruitment and proliferation of cells, required for repairing damaged tissues. Additionally, the immunoblotting analysis revealed an increased expression of neovascularization-related genes, such as *TGF-β1* and *VEGF*, in a BMSC-treated mouse burn injury model.[68] In the present study, group 2 (diabetic rats) displayed the lowest VEGF levels, which might be due to the inability of diabetic rats to properly upregulate *VEGF* expression in response to hypoxia.[20]

Conclusions

Diabetes mellitus exerted detrimental effects on rat tongues. Therapy with BV and BMSCs ameliorated the damaging effects of DM by upregulating the expression of *TGF-β1* and *VEGF*. However, BMSC therapy promoted better results in regenerating the diseased tissues.

Ethics approval and consent to participate

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at Cairo University, Egypt (approval No. CU III F 74 18). This research was conducted in compliance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and regulations (https://arriveguidelines.org). The maintenance and care of the experimental animals conformed with the International Guiding Principles for Biomedical Research Involving Animals.

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

Does the sagittal root position of maxillary anterior teeth affect the decision making on immediate implants in the anterior maxilla? A CBCT-based study

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Abstract

Background. Immediate implant placement in the maxillary esthetic zone is a challenging and demanding task. To achieve favorable results, proper case selection and treatment planning are necessary. Variables like the sagittal root position (SRP) and the labial bone thickness (LBT) of maxillary anterior teeth are of paramount importance for predictable outcomes.

Objectives. The aim of the present study was to evaluate the SRP and LBT of maxillary anterior teeth in the context of immediate implant placement by using cone-beam computed tomography (CBCT) in a sample of the Pakistani population.

Material and methods. A cross-sectional study was conducted using the CBCT scans of patients. The SRP of each tooth (maxillary canine to canine) was evaluated in the sagittal section of a CBCT scan according to the classification by Kan et al. The LBT of each tooth was measured perpendicularly to the long axis of tooth at 3 sites: at the alveolar crest (P1); 2 mm from the alveolar crest (P2); and 4 mm from the alveolar crest (P3). Descriptive statistics were reported for SRP and LBT. The χ^2 test was employed to assess any association between the variables.

Results. Class I SRP was the most prevalent ($n = 196$, 81.7%), while Class III was the least frequent ($n = 1$, 0.4%). The association between the tooth type and SRP was statistically non-significant ($p = 0.510$).

Conclusions. In the evaluated sample of the Pakistani population, the most frequent type of the SRP of maxillary anterior teeth was Class I, which is most favorable for immediate implant placement. Furthermore, the labial bone in the maxillary esthetic zone was found to be mostly thin – LBT was within the range of 0.5–0.9 mm – which makes immediate implant placement in the anterior maxilla a challenge. The results of the present study could serve as a guide for clinicians in terms of appropriate patient selection for immediate implant placement in the maxillary esthetic zone.

Keywords: dental implants, cone-beam computed tomography, maxilla

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Introduction

Dental implants for the replacement of missing teeth have become an increasingly predictable treatment option due to its high rate of clinical success.[1] According to Brånemark's traditional protocol, 12 months are required for adequate bone healing and remodeling before any dental implant can be placed.[2] To overcome this prolonged treatment duration, immediate implant placement has been advocated.[3] The rehabilitation of missing anterior teeth with dental implants in the maxillary esthetic zone poses unique restorative challenges to the dentist. This is due to variability in esthetic outcomes.[4] The conventional approach of delayed implant placement entails the extraction of teeth and waiting for 4–6 months for bone healing.[5] This healing period eventually results in the collapse of soft and hard tissues, leading to a decrease in the tissue volume. To compensate for these deficits, various soft and hard tissue augmentation procedures are required to get a favorable functional and esthetic outcome.[6]

Contemporary literature favors immediate implant placement in the maxillary esthetic zone, as it preserves the existing soft and hard tissue volume.[7] It has many advantages over delayed implant placement, such as a reduction in surgical steps, the preservation of peri-implant soft and hard tissues, and better esthetic outcomes.[3] To achieve optimal esthetic outcomes, proper case selection for immediate implant placement is necessary, which is technically demanding.[1,8] There is a high probability (81%) of the perforation of the labial plate, which could adversely affect esthetic outcomes.[3] Several parameters need to be evaluated prior to immediate implant placement, such as the labial bone thickness (LBT), the root length (RL), the sagittal root position (SRP), the width of keratinized gingiva (WKG), and the gingival biotype.[7,9] Among these factors, SRP and LBT in relation to the surrounding alveolar bone have been shown to have a considerable effect on treatment outcomes.[10] Clinical guidelines recommend an adequate LBT of at least 2 mm to ensure successful treatment.[10,11] In the literature, it has been reported that the volume of the labial bone significantly varies with regard to the root position.[7,8,12] For successful immediate implant placement in the maxillary esthetic zone, the assessment of the root position is mandatory to reduce the risk of treatment failure, such as the perforation of the labial plate, bone dehiscence, etc.[3] A two-dimensional (2D) radiograph cannot provide sufficient information about the root position and the surrounding alveolar bone. Therefore, the use of three-dimensional (3D) imaging, e.g., cone-beam computed tomography (CBCT), is required for the assessment of SRP, LBT and other parameters.[3]

Kan et al. presented a classification for SRP.[7] In this classification, 4 classes are differentiated: Class I – the root is close to the labial cortical plate; Class II – the root is positioned in the middle of the alveolar bone, without nearing the labial or palatal cortical plates at the apical portion; Class III – the root is close to the palatal cortical plate; and Class IV – more than half of the root nears both the labial and palatal cortical plates.[7]

Several studies have evaluated SRP in relation to the alveolar bone.[3,7,11] Kan et al. reported that Class I SRP was the most common (81.1%), followed by Class IV (11.7%), Class II (6.5%), and Class III (0.7%).[7] Similarly, Shrestha et al. used the same classification, and reported that 94.9% of maxillary anterior teeth had Class I SRP, 2.4% had Class II, and 2.7% had Class IV, with no Class III observed.[11]

With the emerging scientific evidence regarding the success of immediately placed implants, the procedure has become the preferred treatment option for both patients and clinicians. To the best of our knowledge, no local study has ever addressed the issue of SRP or LBT in the population of Pakistan. Hence, it is imperative to assess these parameters in the Pakistani population to make proper decisions as to which patient is a candidate for immediate implant placement in the anterior maxilla. This will help clinicians offer predictable outcomes in cases of high esthetic demands. The aim of the present study was to evaluate the SRP and LBT of maxillary anterior teeth in the context of immediate implant placement by using CBCT in a sample of the Pakistani population.

Material and methods

This cross-sectional study was conducted at the Dental Clinics of Aga Khan University Hospital, Karachi, Pakistan, from July 2019 to September 2019. After obtaining an exemption from the institutional Ethical Review Committee (ERC No. 2019-1999-5234), the pre-existing CBCT scans of patients aged 18–60 years, stored in the hospital database, were reviewed. The CBCT scans of the patients having sound bilateral maxillary teeth (canine to canine), fully formed root apices, and with no radiographic evidence of the periapical infection or root resorption of the teeth were included. However, the CBCT scans of the patients who had a history of orthodontic treatment, teeth subjected to peri-apical surgery, alveolar bone deformities, or any pathology, such as a cyst or a trauma, affecting the anterior teeth, as well as distorted images that were not amenable for analysis, were excluded.

The sample size was calculated using a sample size calculator.[13] According to Kan et al., 81% of teeth were characterized by Class I SRP.[7] Keeping this value as an anticipated proportion with a 5% absolute precision and a 95% confidence interval (*CI*), we needed a sample of 237 teeth. As we had to evaluate 6 teeth on each CBCT scan, a total of 40 CBCT scans were needed in the present study.

The patients' demographics, i.e., age and gender, were retrieved using the medical record numbers. All CBCT

scans were obtained with the use of the Orthophos XG 3D Ready/CEPH unit (Dentsply Sirona, Charlotte, USA) operating at 60–90 kV, 3–6 mA, an image volume of 8 cm × 8 cm, a voxel size of 0.2 mm, a scanning time of 14 s, and an exposure time of 2–5 s. The images were saved using the Sidexis XG software, v. 2.63 (Dentsply Sirona). They were viewed using the implant software Galaxis/Galileos Implant Viewer (Dentsply Sirona) on a 27-inch IPS LED-backlit desktop monitor (HP EliteDisplay E271i; Hewlett-Packard, Palo Alto, USA) with a resolution of 1,920 × 1,080 at 60 Hz in a well-lit room. All measurements were taken once by the primary investigator (S.H.).

For the evaluation of SRP, we employed the Kan et al. classification,[7] as shown in Fig. 1 and Fig. 2. To determine the appropriate slice in the sagittal section, the slider tilt tool was used to alter the axial angulation of the image until the entire crown and root portion of the tooth was seen in the sagittal section. Once the appropriate slice was determined, the long axis of the tooth was established by drawing a straight line in the middle of the root. The root position was evaluated according to the classification mentioned above.

The labial bone thickness around each maxillary anterior tooth was measured at 3 points: at the alveolar crest level (P1); 2 mm from the alveolar crest (P2); and 4 mm from the alveolar crest (P3). The measurements were taken perpendicularly to the long axis of the tooth with the help of a measuring tool provided by the software. The SRP and LBT of each maxillary anterior tooth were noted down in the study proforma.

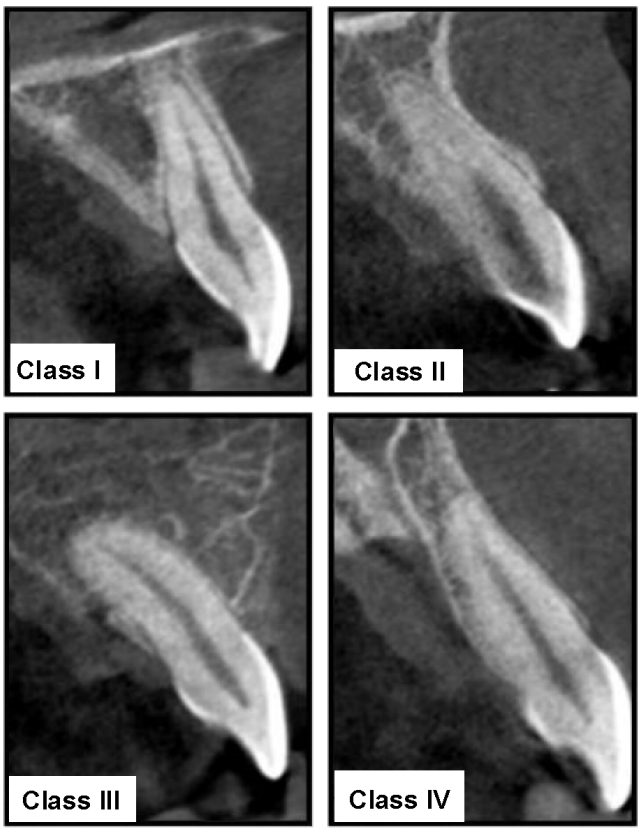

Fig. 1. Classification of the sagittal root position (SRP) of the teeth, as proposed by Kan et al.,[7] for determining the root position of maxillary anterior teeth in the sagittal plane of a cone-beam computed tomography (CBCT) scan

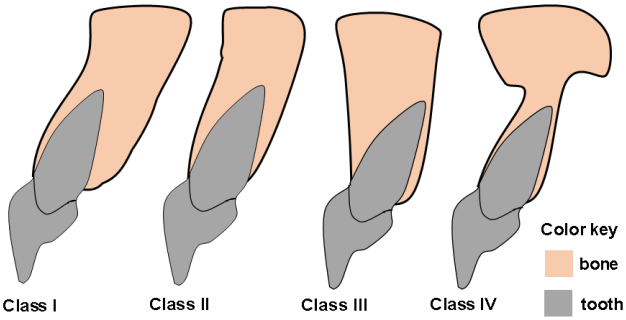

Fig. 2. Graphical presentation of the Kan et al. classification for the sagittal root position (SRP) of anterior teeth[7]

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA). Descriptive statistics, i.e., mean and standard deviation ($M \pm SD$) for the continuous variables (age and LBT), and the frequency distribution (n (%)) for the categorical variable (SRP), were reported. The independent samples t test was applied to compare LBT in both genders and with regard to age. To determine any difference between the right and left sides, the paired samples t test was applied. The χ^2 test was used to establish the association between SRP and the tooth type. A p-value ≤0.05 was set as the level of significance. To assess the inter-examiner reliability, 10 CBCT scans were randomly selected and evaluated by the co-investigator independently. The inter-examiner reliability for the categorical variable SRP and the continuous variable LBT was assessed using Cohen's kappa coefficient (κ) and the intraclass correlation coefficient (ICC), respectively.

Results

A total of 240 maxillary anterior teeth were evaluated for SRP in a sample of the Pakistani population. The mean age of our sample was 30 ±11 years. Class I SRP was the most prevalent (81.7%), while Class III was the least frequent (0.4%). The frequency distribution of SRP is shown in Table 1. Among the tooth types, canines (n = 69/80, 86.3%) were the most frequent in Class I, followed by central incisors (n = 67/80, 83.8%) and lateral incisors (n = 60/80, 75.0%). The association between the tooth type and SRP was statistically non-significant (p = 0.510) (Table 1). The inter-examiner reliability κ statistical values for the categorical variable SRP ranged from 0.42 to 0.90. Similarly, no statistically significant differences in SRP were noted between the 2 age groups and genders.

Table 1. Frequency distribution of the sagittal root position (SRP) among the evaluated teeth of the study participants

SRP	Central incisors	Lateral incisors	Canines	Overall	*p*-value (χ^2 test)
Class I	67 (83.8)	60 (75.0)	69 (86.3)	196 (81.7)	
Class II	8 (10.0)	10 (12.5)	9 (11.3)	27 (11.3)	
Class III	0 (0.0)	1 (1.3)	0 (0.0)	1 (0.4)	0.510
Class IV	5 (6.3)	9 (11.3)	2 (2.5)	16 (6.7)	
Total	80 (100.0)	80 (100.0)	80 (100.0)	240 (100.0)	

Data presented as number (percentage) (*n* (%)).
The Kan et al. classification.[7]

The LBT of each maxillary anterior tooth was measured at 3 sites, i.e., at the alveolar crest level (P1), 2 mm from the alveolar crest (P2) and 4 mm from the alveolar crest (P3). The mean values of LBT are shown in Table 2. For the sake of clinical relevance, we divided the LBT values into 4 categories: <0.5 mm; 0.5–1 mm; 1–2 mm; and >2 mm. The distribution of teeth with regard to the LBT categories is shown in Table 3. The *ICC* values for the continuous variable LBT ranged from 0.67 to 0.94. Statistically significant differences were found between right and left central incisors, lateral incisors, and canines for LBT at all 3 levels.

Table 2. Descriptive statistics of the labial bone thickness (LBT) values [mm] among the evaluated teeth of the study participants

Position	Central incisors		Lateral incisors		Canines	
	right	left	right	left	right	left
P1	0.56 ±0.19	0.67 ±0.21	0.53 ±0.21	0.55 ±0.34	0.58 ±0.28	0.54 ±0.35
P2	0.81 ±0.34	0.89 ±0.32	0.90 ±0.72	0.88 ±0.80	0.89 ±0.54	0.76 ±0.57
P3	0.75 ±0.42	0.85 ±0.37	0.77 ±0.99	0.77 ±1.02	0.80 ±0.50	0.74 ±0.64

Data presented as mean ± standard deviation (*M* ±*SD*).
P1 – alveolar crest level; P2 – 2 mm from the alveolar crest; P3 – 4 mm from the alveolar crest.

Table 3. Distribution of teeth with regard to the labial bone thickness (LBT) categories among the study participants

Teeth	Position	LBT categories			
		<0.5 mm	0.5–1 mm	1–2 mm	>2 mm
Central incisors *n* = 80	P1	26	72	2	0
	P2	8	63	29	0
	P3	6	73	19	2
Lateral incisors *n* = 80	P1	36	59	5	0
	P2	23	39	36	2
	P3	38	43	17	2
Canines *n* = 80	P1	30	63	7	0
	P2	20	52	25	3
	P3	25	53	20	2

Data presented as percentages (%).

Discussion

Immediate implant placement in the maxillary esthetic zone poses its own distinctive challenges.[14] When planning immediate implant placement, a clinician should critically evaluate variables such as the 3D positioning of the root in the alveolar bone, LBT and RL. The results of the present study could serve as a guide for clinicians in terms of appropriate patient selection for immediate implant placement in the maxillary esthetic zone. In this CBCT-based study, we evaluated the SRP and LBT of maxillary anterior teeth in a sample of the Pakistani population. Our study results showed that Class I SRP was the most prevalent, accounting for 81.7% of maxillary anterior teeth. These results are in accordance with a previous study by Sung et al. conducted on the Taiwanese population[3] and research by Kan et al. conducted in the United States.[7] The authors of the abovementioned studies also reported the most prevalent SRP to be Class I, i.e., 87% and 81%, respectively. On the other hand, in the present study, the least common type of SRP was Class III, which was observed in 1 tooth (0.4%) only. Previous studies also reported that Class III SRP prevalence ranged from 0.3% to 0.7%.[3,7] Shrestha et al. reported that 94.9% of maxillary anterior teeth had Class I SRP, 2.4% had Class II, and 2.7% had Class IV, with no Class III observed.[11] However, Xu et al. observed in Chinese adults a higher proportion of buccal-type root positions (95.4%).[15] This may be due to the fact that they used different criteria for the assessment of the root position. Furthermore, no ethnic differences are noted with regard to SRP, as Class I tends to be the most prevalent root position in different ethnicities.

In Class I SRP, the root in its entire length is close to the labial cortical plate, which leaves an adequate amount of bone on the palatal aspect for implant engagement. Thus, it is considered to be one of the most favorable root positions for implant placement, as it spares the labial cortical plate. Moreover, placing an implant in the palatal direction creates a gap between the labial cortical plate and the implant surface called a jumping distance. This jumping distance in the maxillary esthetic zone is of utmost importance, as the labial cortical plate is trabecular and prone to resorption, which can potentially affect esthetic outcomes.[16] Clinical studies recommend placing the bone grafting material in the jumping distance when the distance is >2 mm.[17] On the contrary, Class IV SRP, which comprised 16 (6.7%) of the 240 teeth in our study, is considered a contraindication for immediate implant placement. In this configuration, the root is sandwiched between the 2 cortical plates, thus leaving a minimal amount of bone available for implant integration.[7] Complex hard tissue augmentation procedures are required to get a predictable outcome in such cases. A clinician should be aware of these different root positions and their implications in immediate implant dentistry, and modify the treatment plan accordingly.[18,19]

The labial bone thickness is a prognostic determinant with regard to the outcome of immediate implant therapy in the maxillary esthetic zone. A systematic review by Chen and Buser, comparing the esthetic results of immediate and early implant placement in the anterior maxilla, reported a high frequency of gingival recession in the immediate implant group.[4] The risk factors for gingival recession are bony defects in the labial bone wall, a thin labial cortical plate, a thin gingival biotype, and the labial-palatal malpositioning of the implant.[20] For predictable esthetic and functional outcomes, a minimum LBT of 2 mm is recommended.[21] In our study, the mean LBT for central incisors, lateral incisors and canines ranged from 0.5 mm to 0.9 mm, as shown in Table 2. These findings are in accordance with studies by El Nahass and Naiem,[9] Wang et al.,[1] and AlTarawneh et al.[22] From this, we can infer that a thin labial cortical plate is prevalent in the maxillary esthetic zone. The bone levels at P1 and P2 are crucial for determining the long-term stability of peri-implant soft tissue.[23] With the mean LBT ranging from 0.5 mm to 0.9 mm, the recession of peri-implant soft tissue is anticipated in the long run. This can drastically affect the esthetic outcomes of immediate implant therapy, which is of importance if the esthetic demands of patients are high.[23] Thus, soft and hard tissue augmentation is required in such cases to obtain optimal esthetic outcomes.[18,19] Furthermore, a thin labial bone also precludes the flapless approach for immediate implant placement due to the limited visualization of the underlying bony topography, which may result in the perforation of the facial bone wall.[24]

Cone-beam computed tomography is a common imaging modality used in pre-surgical planning and the visualization of anatomical structures.[11] However, a limitation to all CBCT-based studies is the resolution.[22] A better resolution gives an accurate measurement of the anatomical structures of the area of interest. The resolution of a CBCT scan is inversely proportional to the field of view (FoV). The smaller the FoV, the better the resolution of a CBCT image.[22,25] The CBCT unit used in this study had a medium FoV. The scans were not taken with the aim of using them in the present study; in fact, they were retrieved from the archives of the departmental database.

The present study is the first-ever investigation done on SRP and LBT in the context of immediate implant placement among a sample of the Pakistani population. Longitudinal studies are warranted to establish a temporal relationship between clinical variables and the outcomes of immediate implant therapy in the anterior maxilla.

Limitations

This study was conducted in a single center, which is a potential limitation. The scans included in our study were already stored in the departmental database. The study results can only be generalized to the patients who visited the university hospital, and thus may not be representative of the general population.

Conclusions

Within the limitations of the present study, it is inferred that the most prevalent SRP among the Pakistani subpopulation is Class I, which is favorable for immediate implant placement. However, LBT in the maxillary anterior esthetic zone was in the range of 0.5–0.9 mm, which makes the placement of an immediate implant in the anterior maxilla a challenge.

Ethics approval and consent to participate

Not applicable (an exemption from the institutional Ethical Review Committee at Aga Khan University Hospital, Karachi, Pakistan (ERC No. 2019-1999-5234)).

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

Porosity analysis of four bioceramic materials used for the repair of furcation perforations via micro-computed tomography

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Abstract

Background. Porosity is a crucial parameter that affects the solubility, sealing and mechanical strength of a material. It plays a significant role in determining the success of treatment.

Objectives. The present study aimed to evaluate and compare the porosity of different bioceramic-based materials, using micro-computed tomography (micro-CT).

Material and methods. In the study, 76 permanent lower first or second molars that had been extracted for periodontal reasons and were free of calcification, resorption, root cavities, fractures, or cracks, with discrete roots and complete root apex development were selected. In each of the 4 experimental groups, perforations were made in the furcation areas of 19 molars. Mineral trioxide aggregate (MTA) Angelus®, EndoSequence® Root Repair Material (ERRM), Biodentine™, and BioAggregate were placed on the perforated areas of the samples and scanned with a micro-CT to evaluate porosity. The pore volume and the pore percentage with regard to the closed, open and total porosity of these repair materials were calculated individually in each sample.

Results. While no statistically significant differences were found between group I (MTA), group III (Biodentine) and group IV (BioAggregate) when evaluating the total pore percentage ($p > 0.05$), the parameter in group II (ERRM) was found to be significantly lower as compared to other groups ($p > 0.05$).

Conclusions. In comparison with the other materials used in this study, the use of ERRM may be more suitable for perforation repair.

Keywords: porosity, endodontics, micro-CT, tissue repair, bioceramic materials

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Introduction

Recently, great advances have been made in the biomedical application of ceramics for skeletal repair and reconstruction. Bioceramic materials have been used in both dental implant surgery and endodontic treatment, specifically in the repair of furcation perforations, apexogenesis, apexification, and vital pulp therapy, to induce mineralization.[1] Previously, various endodontic and restorative materials, such as amalgam, glass ionomer cement (GIC), zinc oxide–eugenol cement, 2-ethoxybenzoic acid (EBA), super EBA, gutta percha, composite resin, and Cavit™, have been used for perforation repair. However, due to the lack of biocompatibility of these products and less than ideal prognosis, new materials have beeen investigated.[2] Mineral trioxide aggregate (MTA) is considered the gold standard for various applications, especially as a repair material.[3,4]

Gray ProRoot®, a calcium silicate-based MTA produced by Dentsply Maillefer (Ballaigues, Switzerland), was the only product on the market until white MTA was produced in 2002. Subsequently, new materials have been developed and compared to these 2 gold-standard MTAs. Bioceramic cements have been developed to overcome limitations, such as long hardening duration, difficulty of manipulation, discoloration of dental tissues, low radiopacity, lower compressive strength as compared to dentin, and high costs. These cements have antimicrobial properties, good impermeability and do not contain harmful heavy metals.[5]

Materials used in endodontics must ensure an impermeable cover. This is one of the most important determinants of successful root canal treatment. The durability of perforation repair is affected by the porosity of the material. The presence of closed and open pores is related to the physical properties of the material, and can have an impact on the long-term prognosis. The porosity of the material depends on its water absorption, solubility, permeability, mechanical resistance, and density. The degree of porosity is a critical factor determining its tightness.[6] In previous studies, porosity was evaluated by measuring differences in weight before and after water absorption, using Archimedes' principle, mercury intrusion porosimetry (MIP) and micro-computed tomography (micro-CT).[7–10] Micro-CT evnables a non-destructive and three-dimensional (3D) assessment of dental tissues and of the quality of the material under in vitro conditions.[11] Therefore, in the present study, we evaluated and compared via micro-CT the degree of porosity in the structures of 4 bioceramic-based materials – MTA Angelus®, EndoSequence® Root Repair Material (ERRM), Biodentine™, and BioAggregate – when applied to perforation defects.

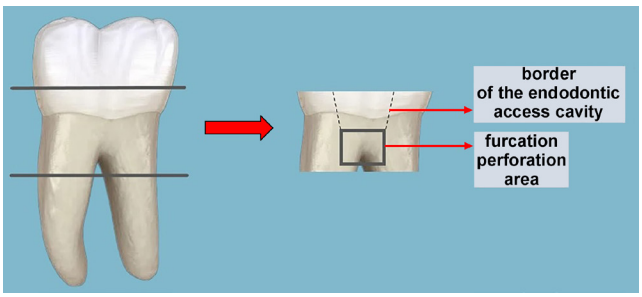

Fig. 1. Schematic pattern of the tooth/root preparation

Material and methods

Specimen preparation

The study used 76 permanent lower first or second molars extracted for periodontal reasons. The teeth were free of calcification, resorption, root cavities, fractures, or cracks. They had discrete roots with complete root apex development.

The organic debris, soft tissues and calculus were meticulously removed from the teeth using the Cavitron 300 Series Ultrasonic Scaling System (Dentsply Sirona, Bensheim, Germany) and a periodontal curette (Gracey #12–14; HuFriedyGroup, Chicago, USA), without causing any damage to the root surfaces or furcation areas. To ensure disinfection, after being stored in 5% NaCl for 1 week, the teeth were kept in a physiological saline solution at ambient temperature until they were used in the study.[12]

The coronal part of the teeth was removed by making 3-millimeter cuts with diamond disks under water cooling. The apical and middle parts of the roots were then removed by cutting. Standard endodontic access cavities were created with a round diamond drill (Hager & Meisinger, Neuss, Germany) and a fissure diamond drill (Hager & Meisinger) under water cooling. The furcation areas of the obtained teeth were perforated with a 1.6-millimeter fissure drill (Hager & Meisinger). To standardize the dentine–cement thickness in the furcation area, we measured the distance between the base of the pulp chamber and the inter-radicular furcation area using a caliper. Only teeth with a thickness of 2–2.5 mm were included in the study. After washing the perforation area with a physiological saline solution, the cavities were dried with a cotton pellet and the outer surfaces of the teeth were meticulously embedded in silicone (Zetaplus C-Silicone; Zhermack, Badia Polesine, Italy), which had been placed inside a plastic cylinder to imitate the peri-radicular tissues, as in a study by Aggarwal et al.[13] The schematic pattern of the tooth/root preparation is presented in Fig. 1.

The samples were randomly divided into 4 experimental groups of 19 specimens each (n = 19). The repair materials used were MTA Angelus (group I), ERRM (group II), Biodentine (group III), and BioAggregate (group IV).

The powder component of MTA Angelus (lot No. 45053; Angelus, Londrina, Brazil) contains tricalcium silicate, bismuth oxide, calcium carbonate, and iron oxide,

while the liquid part is composed of water. Biodentine (lot No. B05663; Septodont, Saint-Maur-des-Fossés, France) consists of tricalcium silicate, small amounts of dicalcium silicate, calcium carbonate, and zirconium oxide. It has a water-based polymer as a liquid component, with calcium chloride, which accelerates the hardening reaction. BioAggregate (lot No. 1201BA; Innovative Bioceramix, Inc., Vancouver, Canada) consists of tricalcium silicate, dicalcium silicate, monobasic calcium phosphate, calcium hydroxide, hydroxyapatite, silica, and tantalum pentoxide. The liquid component is deionized water.[14] EndoSequence Root Repair Material (lot No. BP11001; Brasseler USA, Savannah, USA) contains tricalcium silicate, monobasic calcium phosphate, zirconium oxide, tantalum pentoxide, and filling agents.[15]

The material mixtures were prepared according to the manufacturers' instructions and applied to the perforated areas. To ensure hydration, the access cavities were sealed with Cavit G (3M ESPE, Seefeld, Germany) after placing moisturized cotton pellets in them. Subsequently, the samples were incubated for 72 h at 37°C in an environment with 100% humidity.

Cavit G and cotton pellets were removed from the cavities after the hardening reaction. The samples were removed from the cylinder and the silicone material on their surfaces was cleaned in preparation for micro-CT scanning.

Micro-CT scanning

The samples were enumerated and placed in a Falcon® tube, which was secured to the turntable of the micro-CT device (SkyScan 1172; Bruker, Kontich, Belgium) for high-resolution scanning. The X-ray source of the device was set to 100 kVp and 100 mA, and the samples were beamed with a rotation set at 0.40, using a 0.5-millimeter aluminum(Al)/copper (Cu) filter. Each sample was scanned while rotating 360° for 48 min. Each image was captured for 400 ms, and 3 images were taken from each angle and combined into a single image to reduce the noise ratio. Other adjustments and parameters were chosen based on the manufacturer's recommendations. Raw images were obtained from each sample, using a 10-micrometer pixel scale.

Micro-CT reconstruction and analysis of the samples

The closed pore volume, closed pore percentage, open pore volume, open pore percentage, total pore volume, and total pore percentage of the materials were assessed using micro-CT analysis.

The samples were reconstructed using the NRecon software, v. 1.6.5.2 (Bruker). The parameters for reconstruction were as follows: ring artifact 8; flattening parameter 3; and beam hardening 38%. Contrast adjustments were made according to the instructions provided by the manufacturer. Overall, 1,024 two-dimensional (2D) fractions were obtained from each sample. The raw images were combined using NRecon and fractions in BMP format were obtained for internal structure analysis.

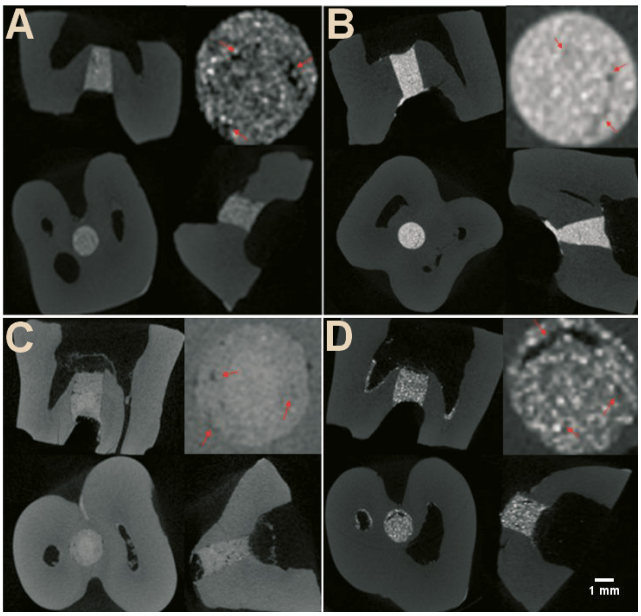

Fig. 2. Images of the coronal, transverse and sagittal planes for all experimental groups

A – group I (mineral trioxide aggregate (MTA) Angelus); B – group II (EndoSequence Root Repair Material (ERRM)); C – group III (Biodentine); and D – group IV (BioAggregate). Red arrows indicate pores in the magnified images in the upper right corner for each group.

Fig. 3. Samples colored using the DataViewer software to enhance the visibility of the pores

A – group I; B – group II; C – group III; D – group IV.

These fractions were then imported into the CTAn software, v. 1.16.4.1 (Bruker), which is used to create quantitative parameters and visual models, and enables densitometric and morphometric measurements. The images were monitored in the X, Y and Z planes using the DataViewer software (Bruker) (Fig. 2,3).

Statistical analysis

The non-parametric Kruskal–Wallis test was performed due to the non-normal data distribution, which was confirmed by the Shapiro–Wilk test. Intergroup correlations were evaluated using the Mann–Whitney *U* test, while the Kruskal–Wallis *H* test with Bonferroni correction assessed intergroup differences. All analyses were conducted using the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA), and a p-value <0.05 was considered statistically significant.

Results

Significant differences in the closed pore volume were found between group II and other experimental groups ($p < 0.05$), while no significant differences were observed between groups I, II and III ($p > 0.05$). Group II showed the lowest values for the closed pore percentage, and the differences was significant, while the highest closed pore percentage values were found in group IV, with the differences being significant ($p < 0.05$) (Table 1).

A statistically significant difference was found between group IV and other experimental groups regarding the open pore volume and percentage, with the BioAggregate group having the lowest values ($p < 0.05$) (Table 1).

Group II had the lowest values for the total pore volume, and the differences with regard to other groups were significant ($p < 0.05$). No statistically significant differences in the total pore volume were observed between groups I, III and IV ($p > 0.05$) (Table 1).

While no statistically significant differences were found between groups I, III and IV for the total pore percentage ($p > 0.05$), group II had the lowest total pore percentage as compared to other groups, and the differences were significant ($p < 0.05$) (Table 1).

Discussion

Due to the complex anatomy of the root canal, perforations may occur at the base of the pulp chamber, or on the coronal, medial or apical parts of the roots during preparation. Various materials, such as GIC, zinc oxide–eugenol cement, calcium hydroxide, and composite resins, have been used for the repair of root perforations. However, none of them have ideal features to meet the characteristics and conditions of root repair. Mercury oscillation and poor esthetics have limited the use of amalgam. Composite resins release toxic monomers and exhibit constriction during polymerization. Other materials are inadequate in terms of impermeability and coating, while having osteogenic, cementogenic and antibacterial effects.[16]

Impermeable materials with high mechanical endurance should be used to repair iatrogenic furcation perforations. The use of bioceramics is considered appropriate in areas not directly related to resistance, particularly for perforation repair and pulp treatment, and in retrograde cavities to ensure hermetic obturation.[17]

Porosity depends on the gaps between the anhydrite cement granules, and is a natural characteristic of bioceramic-based cements. As the hydration reaction progresses, the hydration products fill the gaps, reducing porosity. However, if the water–cement ratio is too high during the mixing process, porosity increases. High liquid–powder ratios may increase porosity and solubility, and the use of a greater amount of liquid increases calcium (Ca) release from MTA.[18] Moreover, the amount of air in the mixture during the mixing process and the pH of the environment also affect porosity.[18,19]

Three different types of pores are observed: closed; through; and blind.[20,21] Closed pores begin and end inside the material, and are therefore not related to the surface or leakage. However, they decrease the mechanical strength of the material.[22] In cements, 10% porosity may reduce mechanical endurance by 50%.[23] Through pores look like tunnels that pass straight from one outer surface of the material to the other. Blind pores start at the outer surface of the material and end inside the material. Through and blind pores are related to the surface, and are called open pores. The presence of open pores negatively affects the adaptation and impermeability of the

Table 1. Comparison of the porosity values between the experimental groups

Group	Material volume [mm³]	Closed pore volume [mm³]	Closed pore percentage [%]	Open pore volume [mm³]	Open pore percentage [%]	Total pore volume [mm³]	Total pore percentage [%]
Group I	4.421	0.251 ±0.025^a	5.68 ±1.23^a	0.226 ±0.046^a	5.11 ±0.14^a	0.477 ±0.017^a	10.79 ±1.02^a
Group II	4.334	0.092 ±0.015^b	2.12 ±0.42^b	0.173 ±0.043^a	3.99 ±0.04^a	0.265 ±0.025^b	6.11 ±0.96^b
Group III	4.525	0.342 ±0.047^a	7.55 ±2.27^a	0.248 ±0.057^a	5.48 ±0.15^a	0.590 ±0.062^a	13.04 ±2.32^a
Group IV	4.624	0.478 ±0.054^a	10.34 ±3.12^c	0.024 ±0.009^b	2.16 ±0.06^b	0.502 ±0.083^a	12.50 ±3.61^a

Data presented as mean ± standard deviation (*M* ±*SD*).
Different letters in superscript show statistically significant differences between the groups.

material.[24] Although porosity has a negative effect on the physical characteristics of the material, it has a positive effect on reactivity and biological characteristics by increasing the release of bioactive ions.[21] The presence of porosity can cause microcracks in the material, and the expansion of these microcracks leads to the deterioration of the structure of the material. Such microcracks cause microleakage. Thus, the functionality of materials changes in the long run.[25]

Gandolfi et al.[10] mixed calcium silicate- and calcium hydroxide-based materials, and found that Biodentine had the highest mechanical endurance due to its low liquid–powder ratio.[26] Camilleri et al. reported that Biodentine was denser and less porous than MTA, but more susceptible to microorganism colonization, which negatively affected its hermetic impermeability.[27] Gandolfi et al. found that the porosity of MTA Angelus decreased over time due to hydraulic expansion and the formation of calcium phosphate, which closes open pores.[28]

Porosity is directly affected by the content of the material, the liquid–powder ratio, and the problems that occur during mixing and handling.[29] In this study, we tested several different materials and analyzed them using micro-CT to obtain 3D, detailed and reliable data on their internal structures.[30] No specific data on the percentage ratios or stable levels of porosity of dental cements, obtained by micro-CT scanning, have been reported in the literature.[31] For this reason, no control group was used in this study, and the porosity measurements of the tested materials were compared with each other.

Two studies evaluated the porosity of bioceramic-based cements. Gonçalves De Souza et al. used micro-CT to evaluate the porosity of iRoot® BP Plus, Biodentine, Ceramicrete, and ProRoot MTA.[29] However, they did not find any significant differences between these materials. Biodentine tended to have the lowest porosity, which was related to the trace amount of water that it contained during the mixing process.[29] Guerrero and Berástegui evaluated only the total porosity of the materials, and found that it was lower in Biodentine as compared to MTA.[32] Silicone blocks were used in both studies. In our study, open, closed and total porosity was calculated separately to provide individual views of the leakage and mechanical endurance of the materials placed inside lower molars.

Our study revealed that ERRM demonstrated significantly lower closed pore volume and percentage as compared to MTA Angelus, Biodentine and BioAggregate. This may be attributed to the fact that ERRM consists of nanoparticles, is prepared as a paste and is easier to handle than other powder–liquid systems. BioAggregate exhibited the poorest results in terms of closed pore volume and percentage. This could be due to the larger particle size as compared to MTA Angelus and Biodentine. As suggested in previous studies, cements with larger particles tend to exhibit more internal porosity when mixed.[27,33] Our results are in line with these findings.

On the other hand, BioAggregate showed significantly better results than MTA Angelus, ERRM and Biodentine for the open pore volume and percentage. The reason why a bioceramic-based cement would exhibit undesirable results for the closed pore volume, but desirable results for the open pore volume can be explained by the fact that materials with higher water absorption tend to expand more. Water absorption may have occurred due to the water present in the dentin tubules and the dentin barrier, rather than the water added during the mixing process. Likewise, BioAggregate shows strong adhesion to the dentin barrier, which is facilitated by humidity absorption in the dentin tubules.[34,35]

The total porosity of ERRM was significantly lower as compared to other materials. When powder and liquid are added separately, and mixed afterward, air bubbles may form during the mixing process, making it harder to obtain a homogeneous mixture and leading to operator errors. EndoSequence Root Repair Material maintains its crystallized structure unless exposed to an acidic environment, absorbing water owing to its crystallized structure and forming a residue similar to hydroxyapatite. The release of calcium ions (Ca^{+2}) reduces porosity.[36–38]

Conclusions

For the treatment to be successful, perforation repair materials need to be durable and leak-proof. Porosity is an important physical feature of bioceramic materials. Our findings indicate that ERRM had significantly lower total porosity than the other materials tested. Within the limitations of this in vitro study, our results can promote further in vivo studies.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Comparison of the angiogenic efficacy of conventional leukocyte- and platelet-rich fibrin versus low-speed advanced platelet-rich fibrin: An in vitro chorioallantoic membrane assay study

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Abstract

Background. Platelet-rich fibrin (PRF) is widely used in periodontics for its wound healing potential. Two major variations of PRF are the original leukocyte- and platelet-rich fibrin (L-PRF) and the modified low-speed advanced PRF (A-PRF).

Objectives. The aim of the present study was to evaluate and compare the conventional L-PRF protocol and the low-speed A-PRF protocol in terms of angiogenic potential of PRF, using an in vivo chorioallantoic membrane (CAM) assay.

Material and methods. Fifteen fertile Giriraja eggs were procured and after a 3-day incubation period, randomly allotted into 3 groups: control; L-PRF; and A-PRF. A total of 20 mL of blood was collected from systemically healthy male volunteers aged 18–24 years, using a standard protocol. The PRF samples were inoculated on the CAM of the eggs. On the 10th day, the eggs were reopened and photographed. The parameters assessed were the number, length, size, and density of blood vessels, as well as the number of junctions formed. The photographs were analyzed using the ImageJ and ProgRes® CapturePro software.

Results. Seven days after inoculation, both the A-PRF and L-PRF groups exhibited significantly better results than the control group in terms of the number (59.20 ±6.61 vs. 48.80 ±5.07 vs. 19.20 ±6.98), length (25,000 ±1,813.10 µm vs. 17,000 ±282.90 µm vs. 8,000 ±184.49 µm), size (230,000 ±15,054.00 µm^2 vs. 200,000 ±8,295.27 µm^2 vs. 150,000 ±4,105.16 µm^2), and density (central: 9,100 ±296.78 vs. 5,370 ±272.42 vs. 1,420 ±564.36; peripheral: 9,094 ±400.14 vs. 3,370 ±479.39 vs. 5,420 ±746.73) of blood vessels, as well as the number of junctions formed (52 ±3.81 vs. 41 ±1.58 vs. 33 ±4.64), respectively.

Conclusions. The angiogenic potential was increased by the exposure to both L-PRF and A-PRF. However, A-PRF demonstrated statistically significant benefits in terms of the number, length, size, and density of blood vessels, as well as the number of junctions formed in comparison with the control and L-PRF groups.

Keywords: angiogenesis, wound healing, platelet-rich fibrin, chorioallantoic membrane assay

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Introduction

Chronic periodontitis inevitably leads to tissue loss or damage, making the restoration of tissues a challenging task due to the difficulty in repairing and regenerating the periodontium. Therefore, seamless wound healing is a crucial factor in determining the success of periodontal therapy.[1]

The literature describes various techniques regarding the stimulation of the wound healing process, either naturally or artificially.[2] The artificial pathway involves the use of chemical agents, such as hormones (e.g., human growth hormones, insulin and testosterone), or physical means, such as photobiomodulation and heat therapy. The natural pathway is accelerated by using autologous materials, such as platelet concentrates, or herbal extracts, like green tea or the kiwi extract. The selection of the technique depends on the size and location of the tissue to be regenerated, the clinician's expertise, patient preference, affordability, and the feasibility of the required surgical procedure.[3]

Regenerative periodontics and tissue engineering aim to restore both the structure and function of the damaged tissues. These emerging approaches employ specific biocompatible and bioactive composites or natural scaffolds, into which cells or bioactive molecules are incorporated to construct a dynamic environment for wound healing within the damaged tissues. Several recent studies have focused on autologous platelet concentrate derivatives, which may delay complications and boost tissue regeneration. Platelet derivatives were first introduced by Choukroun et al. in 2001,[4] and several modifications of the original protocol are currently in use. Platelet-rich fibrin (PRF) is a second-generation platelet concentrate obtained by centrifuging the patient's blood without any external additives, such as anticoagulants. Currently, low-speed advanced PRF (A-PRF) and conventional leukocyte- and platelet-rich fibrin (L-PRF) are popular for various dental applications, including the treatment of necrotic tissue, like pulp and interdental papillae, through pulp revascularization and papilla regeneration, in ridge augmentation, orofacial reconstruction, and the repair of an oro-antral fistula, and intrabony or furcation defects. They can also be used as a bandage over soft tissue donor sites and for recession coverage.[3–6]

The major rationale for the use of PRF is that angiogenesis is the cornerstone of all biochemical processes in our body. A few studies have quantified the level of vascular endothelial growth factor (VEGF), but none has directly compared the angiogenic potential of different types of PRF or various PRF protocols. Since the angiogenic properties of the biomaterial contribute immensely to its wound healing potential, the present study may help clinicians use the available resources wisely and judiciously, thereby reducing the cost of additional regenerative materials.[7]

The study aimed to evaluate and compare the conventional L-PRF protocol and the low-speed A-PRF protocol in terms of angiogenic potential of PRF, using an in vivo chorioallantoic membrane (CAM) assay.

Material and methods

This controlled in vitro study was approved by the Institutional Review Board at Bapuji Dental College and Hospital, Davanagere, India (approval No. BDC/509/2019-20), and was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2000. Blood samples were collected from systemically healthy male volunteers aged 18–24 years. Volunteers were excluded from participating in the study if they were heavy smokers or on drug therapy that might affect the outcomes of the study. Three groups were created: control; L-PRF; and A-PRF. Two different types of PRF were prepared, i.e., L-PRF was prepared using the standard protocol of 2,700 rpm for 12 min,[8] while A-PRF was obtained by centrifuging blood at 1,500 rpm for 14 min.[9]

The samples were inoculated on the CAM of fertile Giriraja eggs. The assay was conducted randomly in pentaplicate, and the eggs were reopened on the 10th day of incubation to record the results. Photographs were taken on the 3rd and 10th day, and the images were analyzed using the ImageJ 1.53k software (https://imagej.net/ij) bundled with the Java analysis software, v. 1.8.0_172,[10] and the ProgRes® CapturePro software, v. 2.8.8 (Jenoptik, Jena, Germany).[11] The parameters assessed included the number, length, size, and density of blood vessels, as well as the number of junctions of blood vessels.

Platelet-rich fibrin preparation

Approximately 20 mL of venous blood was collected from each volunteer, using a needle and a sterile plastic vacutainer tube for the preparation of PRF. The blood samples were transferred into 2 sterile 10-milliliter glass tubes without anticoagulation, one for L-PRF and the other for A-PRF. The tubes were immediately centrifuged (PRF Duo Quattro; Ostralos Ltd, Auckland, New Zealand), using a standard protocol.

Platelet-rich fibrin was prepared by a single investigator according to the protocol described by Dohan et al. in 2004[8] for L-PRF and Ghanaati et al.[9] for A-PRF. Leukocyte- and platelet-rich fibrin was obtained by centrifuging 10 mL of blood in a red-capped L-PRF tube at 2,700 rpm for 12 min, while A-PRF was prepared by centrifuging 10 mL of blood in a red-capped A-PRF tube at 1,500 rpm for 14 min. In both cases, 3 layers were obtained: platelet-poor plasma (PPP); a PRF clot; and a red blood cell (RBC) layer. The fibrin clot was easily separated from RBCs at the bottom. For both types of PRF, 5 samples were prepared.

Chorioallantoic membrane assay

The CAM assay was conducted at the Government Veterinary Hospital in Bengaluru, India.

The study was carried out according to the standard protocol[12] and involved the following steps:

1. Obtaining fertile eggs: 15 fertile Giriraja chicken eggs, weighing approx. 58 g each, incubated for 72 h at 37°C and 70–80% humidity, were obtained from the egg hatchery at the Department of Poultry, Government Veterinary Hospital, Bengaluru, India;
2. Incubation: The eggs were incubated in the Multiquip E2 incubator (Multiquip, Sydney, Australia) at 37°C and 60% humidity. The egg tray was automatically tilted by 45° every 30 min to simulate the natural process;
3. Disinfection: The eggshells were disinfected with a 70% ethanol solution for 2–3 min (Fig. 1);
4. Candling: Candling of the embryos was performed to confirm egg fertility and determine the position of the air sac (Fig. 2), thus establishing the optimal position for the placement of the biomaterial on CAM. A pencil was used to mark the opening area;
5. Opening: On day 3 of chick embryo development, a small opening was made in the shell under aseptic conditions, using a wheel bur and a blunt tweezer (Fig. 3);
6. Inoculation: The eggs were divided into 3 groups: control; L-PRF; and A-PRF. Both L-PRF and A-PRF were cut into uniform fragments measuring 1 mm × 2 mm, and were carefully inoculated on the CAM of the eggs over the blunt end of the egg, where the opening was made (Fig. 4);
7. Sealing: The opening was resealed with paraffin wax and the eggs were returned to a mini-incubator for the next 7 days (Fig. 5);
8. Reopening: After disinfecting the eggs with a 70% ethanol solution, they were dewaxed manually using a hot instrument. The images were taken after the contents of the eggs were transferred to a Petri dish together with CAM (Fig. 6).

Fig. 1. Disinfecting the egg with a 70% ethanol solution

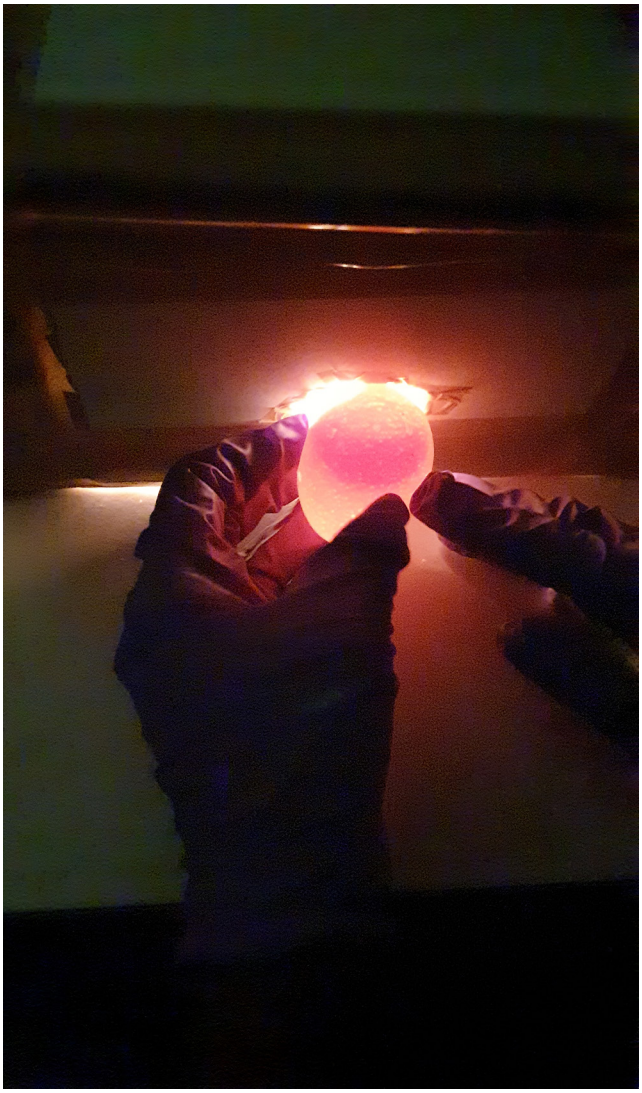

Fig. 2. Candling of the embryos to determine the position of the air sac

Fig. 3. Opening the eggshell with a wheel bur and a blunt tweezer

Fig. 4. Inoculation of different types of platelet-rich fibrin (PRF)

Fig. 5. Sealing of the opening with paraffin wax and incubation

Fig. 6. Reopening the eggshell on the 10th day of incubation and transferring its contents to a Petri dish together with the chorioallantoic membrane (CAM)

The obtained images were analyzed to determine the effects of the biomaterial on the angiogenesis process in the CAM of the developing chick embryo.

Image analysis

After applying the Mexican Hat Filter, conversion to 8-bit and the measurement of the vessel area, the density of the vascular network was quantified using the ImageJ software.[13]

Morphometric analysis was carried out using the ProgRes CapturePro software. For vascular morphometric analysis, the images were captured using a 64MP wide-angle primary digital camera (Samsung Galaxy F62; Samsung Electronics Co., Ltd., Suwon, South Korea), set at F1.8, 1/50 s, 5.23 mm, ISO 200, with auto white balance and no flash. The measurements were made using the ProgRes CapturePro software. To ensure accuracy, we calibrated the magnification, using a stage micrometer before measurement. The images were saved and recalled on the monitor. All measurements were taken using the software measuring tools.

Stage readings were reviewed for reassessment. The number of vessels was measured in each group, and black arrows were used to mark recognizable vasculature. The length of the total vasculature was measured in micrometers, the size of the vessels was recorded in micrometers squared and the number of junctions of blood vessels was calculated using the ProgRes CapturePro software by counting the total number of branch points. The density of blood vessels was determined using the ImageJ software (v. 1.38) by measuring the amount of red pixels per area unit (Fig. 7).

The analysis was conducted by 2 independent observers to minimize subjectivity.

Statistical analysis

The obtained results were tabulated and subjected to statistical analysis. The mean and standard deviation ($M \pm SD$) values were calculated for all parameters. The one-way analysis of variance (ANOVA) was used to compare the results obtained for the replicas within the groups. The Bonferroni test was used to analyze differences between the groups for multiple comparisons of each parameter. A p-value <0.05 was considered statistically significant.

Results

All images were analyzed using 2 software programs – ImageJ and ProgRes CapturePro. No complications, such as embryo death, contamination or inclusion bodies, were

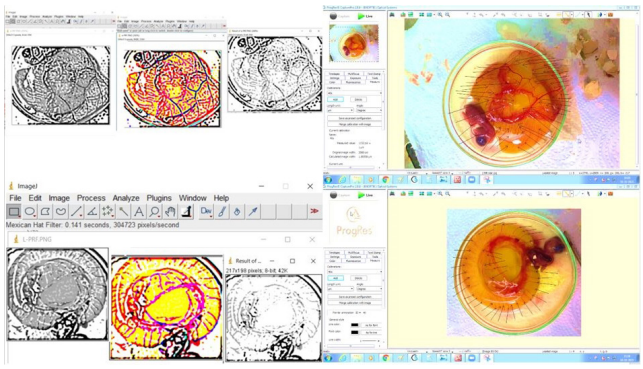

Fig. 7. Image analysis with the use of the ImageJ and ProgRes CapturePro software

observed during the study period. Both the A-PRF and L-PRF groups showed a significant increase in the number and density of blood vessels ($p < 0.01$) as compared to the control group (Table 1). In the peripheral zone, a small but dense vasculature spread was noted in the L-PRF group. In contrast, the A-PRF group exhibited more apparent and larger vessels, which were densely distributed throughout the tissues. The branched networks in the A-PRF group were also large in size and showed a high density (Table 1). Both the A-PRF and L-PRF groups showed a significant increase in the length of blood vessels as compared to the control group ($p < 0.001$). The A-PRF group presented a greater length of blood vessels than the L-PRF group (Table 1). Additionally, the A-PRF group had a significantly greater size of blood vessels than the L-PRF and control groups (Table 1). The A-PRF group also demonstrated an increased number of junctions as compared to the control and L-PRF groups (Table 1).

Discussion

Healing involves the restoration of both quantity and quality of healthy tissues through regeneration and repair. Angiogenesis is a fundamental concept in all physiological as well as pathological events in a biological system. Disturbances in angiogenesis may result in cancer, rheumatoid arthritis, psoriasis, retinopathies, and obesity, when it is increased, and in ulcers, chronic wounds, stroke, and even coronary artery disease, when it is decreased.[14] Our study focused on finding an ideal biomaterial to promote wound healing, with good predictability, reduced surgical time and minimal morbidity.[15]

Autologous platelet concentrates are an ideal option, as they contain concentrated autologous growth factors that stimulate stem cells, attract them to the injured site, stimulate angiogenesis, enhance immunity, act as a scaffold, and encourage wound healing. The use of PRF has been frequently reported in the literature with regard to regeneration. Platelet-rich fibrin offers several benefits, including antibacterial efficacy, root conditioning properties and recession coverage. It is used for the treatment of the chronic ulcers caused by diabetes or burns. Platelet-rich fibrin also promotes osteogenesis through its osteoconductive efficacy, although its osteoinductive properties have not yet been established.[16]

Table 1. Results obtained in the different groups for the parameters measured

Parameter	Control group	L-PRF group	A-PRF group
Blood vessels *n*	19.20 ±6.98	48.80 ±5.07*	59.20 ±6.61*#
Central density	1,420 ±564.36	5,370 ±272.42*	9,100 ±296.78*#
Peripheral density	5,420 ±746.73	3,370 ±479.39*	9,094 ±400.14*#
Total length [µm]	8,000 ±184.49	17,000 ±282.90*	25,000 ±1,813.10*#
Total size [µm^2]	150,000 ±4,105.16	200,000 ±8,295.27*	230,000 ±15,054.00*#
Junctions *n*	33 ±4.64	41 ±1.58*	52 ±3.81*#

L-PRF – leukocyte- and platelet-rich fibrin; A-PRF – advanced PRF; * significantly different from the control group ($p < 0.01$); # significantly different from the L-PRF group ($p < 0.01$).

Leukocyte- and platelet-rich fibrin is a second-generation platelet concentrate, introduced by Dohan et al. in 2004.[8] In an in vitro study, the use of L-PRF resulted in a very strong stimulation and proliferation of endothelial cells, pericytes, fibroblasts, and pre-keratinocytes for more than 28 days.[17] In another study, it was found that the growth factor release profile of L-PRF was up to 7 days.[18] For such reasons, L-PRF is widely used in the treatment of periodontal defects, as well as in systemic applications for diabetic foot ulcers, venous ulcers and others.[17,18]

In 2014, Ghanaati et al. developed a new protocol concept for a low centrifugation speed.[9] It was based on the fact that a low speed helps in the even distribution of platelets, increases their amount and results in greater leukocyte entrapment throughout the fibrin clot. The protocol was named A-PRF.[19,20] Histological and biochemical studies revealed that A-PRF was more porous, heavily packed with monocytes and platelets, and uniformly saturated with growth factors. Moreover, it was shown that this type of PRF had a higher growth factor release profile for up to 10 days as compared to L-PRF.[21]

Recent research and clinical trials have concluded that the superior healing properties of L-PRF and A-PRF are related to their chemoattractive, angiogenic, osteogenic, anti-inflammatory, anti-microbial, pain-inhibitory, and wound healing characteristics.[22] Various studies have shown improved bone regeneration and soft tissue regeneration when using these biomaterials, with or without bone grafts.[23] However, angiogenesis is a complex procedure that involves a sequential interplay of various cells, growth factors and environmental factors.[24] The aim of this study was to evaluate the angiogenic efficacy of conventional L-PRF vs. low-speed A-PRF, as there is a lack of literature directly comparing the in vivo angiogenic potential of these 2 commonly used PRF protocols.

The chick CAM assay is one of the oldest and most widely used methods for studying angiogenesis in vivo. It was developed by Folkman in 1974,[25] and takes advantage of the fact that CAMs are present in the fertile eggs of all avian species, they are immunodeficient and contain numerous blood vessels. This structure rapidly expands, generating a rich vascular network that enables the examination of tissue grafts, tumor growth, wound healing, drug delivery, and angiogenic and anti-angiogenic molecules, as well as toxicological analysis. These characteristics are ideal for in vivo assays. The method is reproducible, fast, suits large-scale screening, and allows the simple visual-

ization of new vascularization under a microscope. Also, this assay is one of ethically acceptable methods of investigating angiogenesis in vivo.[26,27]

We selected the CAM assay as the model to study angiogenesis due to its abovementioned benefits. The eggs used in the study were procured from the same hatchery and were at the same stage of incubation to minimize bias. In a recent in vivo-in vitro randomized controlled trial on the effect of adding PRF to 3 different types of porcine collagen membranes (mucoderm®, collprotect® and Jason®), the CAM assay was selected as the in vivo model to study angiogenesis.[27]

Miron et al. conducted a study on male patients aged 20–40 years to prepare PRF from blood samples and eliminate bias based on the relationship between age, gender and healing potential.[28] Smoking and nicotine have negative effects on epithelial cell proliferation and connective tissue interaction, which are essential steps in wound healing. Therefore, we excluded chronic smokers from our study protocol.[29] Additionally, systemically compromised patients, such as those with bleeding disorders, diabetes mellitus, or patients on drug therapy that might affect the outcomes of the study were excluded.

All parameters, including the number of blood vessels formed, the total vasculature length, the blood vessel size, the vascular network density, and the number of junctions of blood vessels, were analyzed using 2 software programs, ImageJ[11,12] and ProgRes CapturePro,[30]after processing the images taken at the end of the 10th day. Blood vessel count was performed both quantitatively and qualitatively.

During the qualitative analysis, we observed strong neovascularization in the A-PRF group and moderate neovascularization in the L-PRF group as compared to the control group. Both the L-PRF and A-PRF groups showed significantly higher blood vessel formation, greater density, increased length, and larger size of blood vessels as compared to the control group. A recent in vivo-in vitro study compared the angiogenic efficacy of PPP, platelet-rich plasma (PRP) and PRF.[31] The concentrations of angiogenic factors and their bioactivity were determined, and the results showed that in the PRP and PRF preparations, both VEGF and platelet-derived growth factor BB (PDGF-BB) were significantly more concentrated than in PPP, whereas PRF was the most effective for wound closure. In the CAM assay, the PRF membranes were the most effective for neovascularization.[31] The results of previous studies[31,32] are in agreement with our outcomes, as L-PRF and A-PRF demonstrated significant neoangiogenesis both quantitatively and qualitatively. Another study was conducted to evaluate the angiogenic potential of L-PRF using in vitro and in vivo assays.[33] The in vitro assay utilized an antibody array to determine the growth factors released by L-PRF. High levels of CXC chemokine receptor 2 (CXCR-2) ligands and epidermal growth factor (EGF) were reported. The in vivo study was conducted using a CAM assay. It was found that L-PRF induced in vitro the key steps of the angiogenic process, including endothelial cell proliferation, migration and tube formation, thus accelerating angiogenesis.[33] However, no such study has been conducted for A-PRF.

Advanced PRF showed a significantly higher blood vessel density, centrally and peripherally, longer blood vessels, more junctions, and larger blood vessels than both the control group and the L-PRF group ($p < 0.05$). In a recent study, the release of growth factors such as PDGF-AA, transforming growth factor beta 1 (TGF-β1), VEGF, EGF, and insulin-like growth factor 1 (IGF-1), was assessed; it was found that the release of VEGF for L-PRF and A-PRF on the 1st day was 106 pg/mL and 150 pg/mL, and on the 10th day, it was 175 pg/mL and 210 pg/mL, respectively.[32] The study reported that A-PRF released more growth factors than L-PRF, indicating that the low-speed concept is more effective.[32] The low-speed concept leads to a more even distribution of platelets and growth factors throughout the clot matrix, unlike the conventional protocol, where most growth factors concentrate just above the RBC layer.[33] Therefore, it can be concluded that reducing the centrifugation speed significantly enhances angiogenesis. Our study also confirms the superior performance of A-PRF, which can be attributed to an increased diffusion and dispersion of growth factors from A-PRF as compared to L-PRF.

Overall, the results of our study demonstrate that both A-PRF and L-PRF have strong angiogenic properties. The limitations of the present study include the absence of histological and immunological evaluations.[34] However, the present study provides new insights with regard to the future of angiology and regenerative periodontics.

Conclusions

Exposure to both L-PRF and A-PRF increased the angiogenic potential. Advanced PRF demonstrated a statistically significant enhancement in the number, length, size, and density of blood vessels, as well as in the number of junctions of blood vessels. Further in vivo and in vitro studies using different models of angiogenesis are recommended to determine the suitability of these materials as ideal wound healing agents.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board at Bapuji Dental College and Hospital, Davanagere, India (approval No. BDC/509/2019-20).

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

Effects of different concentrations of bromelain and papain enzymes on shear bond strength of composite resin to deep dentin using an etch-and-rinse adhesive system

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Abstract

Background. The dentin substrate can be modified by proteolytic agents, which may affect the bonding strength of adhesive systems to the treated dentin surface. Papain, a cysteine protease enzyme with antibacterial and anti-inflammatory properties, can be used for deproteinization of dentin. An alternative deproteinizing enzyme is bromelain.

Objectives. This study aimed to evaluate the impact of deproteinization on the shear bond strength (SBS) of composite resin to deep dentin using different concentrations of bromelain and papain.

Material and methods. Sixty upper premolars were extracted and randomly divided into 5 groups ($n = 12$ per group). In all groups, the dentin surface was etched with 37% phosphoric acid. Group 1 did not receive any enzyme treatment, group 2 was treated with a 10% papain solution, group 3 was treated with a 15% papain solution, group 4 was treated with a 6% bromelain solution, and group 5 was treated with a 10% bromelain solution. After applying an etch-and-rinse adhesive system, the specimens were restored with composite resin and the SBS was measured.

Results. Statistically significant differences were found between groups 2 and 3 (10% papain and 15% papain, $p = 0.004$), groups 2 and 4 (10% papain and 6% bromelain, $p = 0.017$), groups 4 and 5 (6% bromelain and 10% bromelain, $p = 0.021$), and groups 3 and 5 (15% papain and 10% bromelain, $p = 0.005$).

Conclusions. Deproteinization with papain and bromelain at different concentrations after acid etching did not affect the SBS of composite resin to deep dentin when using an etch-and-rinse adhesive system. However, the group deproteinized with 15% papain demonstrated a higher SBS than the group deproteinized with 10% papain, and the group deproteinized with 6% bromelain showed a higher SBS compared to the group deproteinized with 10% bromelain.

Keywords: shear bond strength, papain, bromelain, dentin

Introduction

The mechanism of bonding to dentin is based on the hybridization concept. In etch-and-rinse adhesive systems, acid etching agents are applied to the dentin surfaces to remove the smear layer, demineralize the dentin and expose the collagen fibril network. Adhesion occurs through the diffusion of adhesive monomers into the exposed collagen layer and subsequent formation of the hybrid layer.[1–4] However, excessive dehydration can cause the collapse of the collagen fibril network, reducing the infiltration of monomers into deeper areas and increasing the risk of adhesive failure. On the other hand, excess water prevents penetration and polymerization of the bonding systems.[5,6]

Improving the physical properties of the bonding agent or modifying the dentin substrate itself can enhance dentin bonding.[7–10] In a process known as dentin deproteinization, proteolytic agents are used to modify acid-etched dentin and eliminate the organic content of the dentin substrate. Deproteinizing solutions modify the dentin surface by dissolving exposed collagen fibrils, leading to greater exposure of the dentinal tubules. This results in dentin that is more similar to etched enamel, which has promising characteristics for promoting adhesion. This type of surface has shown multiple irregularities, with good mechanical retention of the adhesive in the modified dentin substrate.[11–13]

Pre-treatment with proteolytic enzymes has been recommended to achieve better adhesion to dentin.[8,11] Papain is a proteolytic enzyme that removes caries without damaging surrounding tissues. It is extracted from the ripe fruit of *Carica papaya*, a member of the Caricaceae family. The enzyme is a cysteine protease that has demonstrated antibacterial and anti-inflammatory properties.[8,14] It has been reported that the use of 10% papain as a deproteinizing agent before acid etching increases subsequent bond strength by removing organic elements.[15] Another related study showed that the highest bond strength values of orthodontic brackets bonded with resin-modified glass ionomer cement (RMGIC) were attained after enamel deproteinization with 8% and 10% papain, which were more effective than lower concentrations of the enzyme (2%, 4% and 6%).[14]

Bromelain is a deproteinizing enzyme commercially extracted from the fruit or stem of the pineapple.[16] It has been shown to improve the bond strength when applied after acid etching of dentin.[11] However, no study has compared the effects of different concentrations of bromelain and papain enzymes on the shear bond strength (SBS) of composite resin to deep dentin. The aim of this investigation was to evaluate the effects of treatment with different concentrations of enzymes on the SBS of composite resin to deep dentin using an etch-and-rinse adhesive system. The null hypothesis states that there is no correlation between the application of different concentrations of bromelain and papain enzymes as dentin pre-treatments and the SBS of subsequent composite bonding to deep dentin.

Material and methods

Specimen preparation

The Ethics Committee of Shiraz University of Medical Sciences approved the research protocol (approval No. IR.SUMS.REC.1396.S509). For this in vitro experimental study, we collected 60 extracted human upper premolars that were free of caries, restoration or cracks. The teeth were washed under running water to remove residual debris and tissue, and stored in a 0.1% thymol solution at 4°C for 1 week. Afterwards, the teeth were thoroughly washed with tap water and embedded in self-cure acrylic resin (Acropars; Marlik Co., Tehran, Iran) up to the cement–enamel junction. The occlusal surface was positioned parallel to the acrylic resin surface, making it ready for experimental surface preparation. The occlusal thirds of the crowns were sectioned perpendicular to the long axis of the tooth using a water-cooled, low-speed cutting machine (Mecatome T201 A; Presi, Grenoble, France) to remove the occlusal enamel and superficial dentin, and obtain flat, deep dentin surfaces. To polish the superficial dentin and create a uniform smear layer, we applied 600 grit silicon carbide paper to the prepared surfaces. Papain powder (Organika, Vancouver, Canada) and bromelain enzyme powder (Biozym Scientific GmbH, Olendorf, Germany) were weighed using a balance with an accuracy of ±0.1 mg (GR-300; A&D Company Ltd., Tokyo, Japan), and added to distilled water to achieve different concentrations of these enzymes. Specifically, 10 g and 15 g of papain powder were added to 100 mL of distilled water to prepare 10% and 15% papain solutions, respectively. Additionally, 6 g and 10 g of bromelain powder were added to 100 mL of distilled water to achieve concentrations of 6% and 10%, respectively. The 60 teeth were randomized into 5 groups (n = 12 per group). Each group was assigned to a different method of dentin pre-treatment.

For group 1, the dentin surface was etched with 37% phosphoric acid (DenFil; VERICOM Co., Ltd., Chuncheon, Korea) for 15 s, then rinsed with distilled water for 10 s and blot dried. For groups 2 and 3, the dentin surface was etched with 37% phosphoric acid for 15 s, rinsed with distilled water for 15 s, and blot dried with a cotton pellet to remove excess water. The surface was then treated with 10% papain (group 2) or 15% papain (group 3) for 60 s, washed with distilled water for 15 s and blot dried. For groups 4 and 5, the dentin surface was etched with 37% phosphoric acid for 15 s, rinsed with distilled water for 10 s and blot dried. Subsequently, the dentin surface was treated with 6% bromelain (group 4)

or 10% bromelain (group 5) for 60 s, washed with distilled water for 15 s and blot dried.

After preparing the dentin surface, composite resin was bonded to 10 specimens in each group for SBS testing. The remaining 2 specimens in each group were prepared for evaluation using scanning electron microscopy (SEM), as described below.

Shear bond strength testing

An etch-and-rinse system (Adper Single Bond 2; 3M ESPE, St. Paul, USA) was applied to the treated dentin surfaces, according to the manufacturer's instructions. A light-emitting diode (LED) polymerizing unit (Bluelex GT-1200; MONITEX Industrial Co., Ltd., New Taipei City, Taiwan) with a wavelength of 470 nm and a light intensity of 1,200 mW/cm^2 was used for curing. Subsequently, a plastic mold with a height of 2 mm and an internal diameter of 3 mm was placed over the prepared dentin surface. A 2-mm thick increment of composite resin (Filtek™ Z350; 3M ESPE) was inserted into the mold and light cured for 40 s from the occlusal direction. The mold was removed and the specimens were stored in distilled water at 37°C for 24 h in an incubator (ES 252; NÜVE, Ankara, Turkey) before testing. The specimens were individually transferred to the universal testing machine (Z020; ZwickRoell, Ulm, Germany) and subjected to SBS analysis at a crosshead speed of 1 mm/min. The experimental design used in this study is presented in Fig. 1. Figure 2 shows a prepared specimen transferred to the universal testing machine.

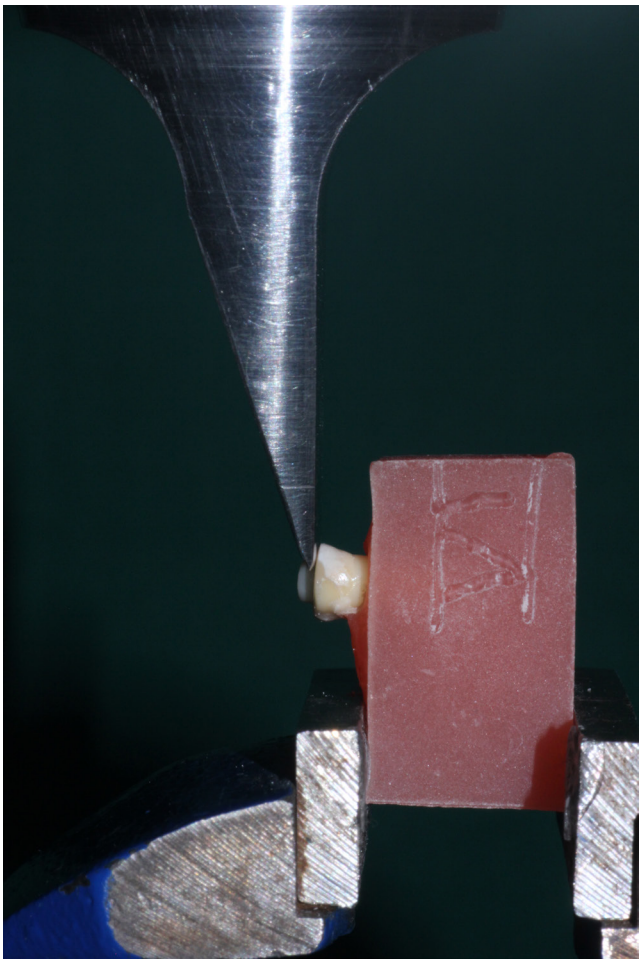

Fig. 2. Sample prepared and transferred to the universal testing machine for measuring the shear bond strength

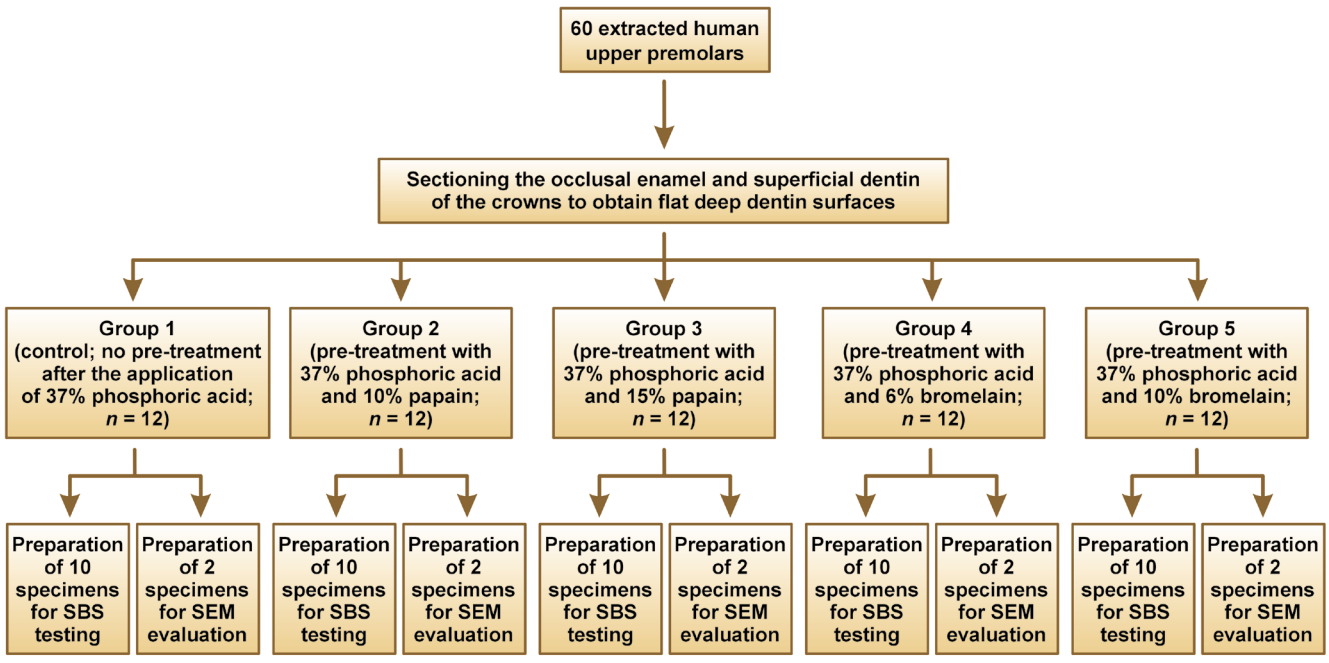

Fig. 1. Schematic diagram of the study design

SBS – shear bond strength; SEM – scanning electron microscopy.

Scanning electron microscopy evaluation

Two specimens from each group were evaluated using a scanning electron microscope (KYKY-EM3200; KYKY Technology Co. Ltd., China). The specimens were initially sectioned using a diamond disc to obtain dentin samples with a thickness of 2 mm. These samples were then dried in a desiccator for 24 h, sputter-coated with gold and examined under the microscope at 2 different magnifications (×3,000 and ×4,000).

Statistical analysis

Statistical analysis was conducted using SPSS for Windows software, v. 16 (SPSS Inc., Chicago, USA), with data analysis performed using one-way analysis of variance (ANOVA) followed by Tukey's test. The significance level set for the study was $p < 0.05$.

Results

The descriptive statistics of the experimental SBS values for all groups, including the mean (*M*), standard deviation (*SD*), and minimum and maximum values, are presented in Table 1. Moreover, the *M* and *SD* values of the SBS for all groups are shown in Fig. 3. One-way ANOVA revealed statistically significant relationships between the experimental groups ($p < 0.05$). The study results indicate that group 3 (37% phosphoric acid + 15% papain) and group 4 (37% phosphoric acid + 6% bromelain) had the highest mean SBS values compared to the other experimental groups.

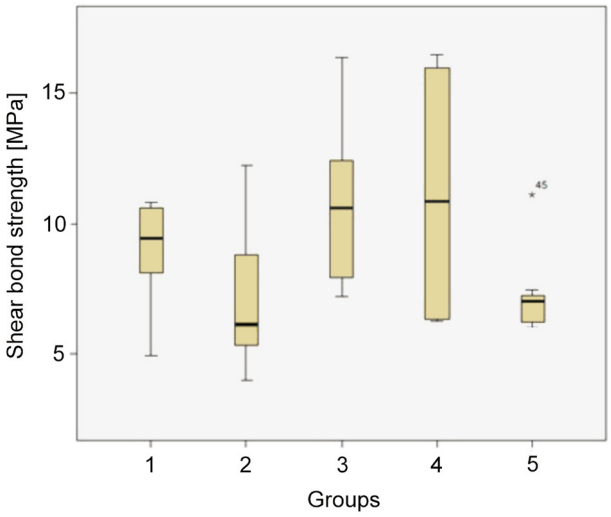

Fig. 3. Shear bond strength values in the experimental groups
group 1 – the control group etched with 37% phosphoric acid; group 2 – treated with 37% phosphoric acid and 10% papain; group 3 – treated with 37% phosphoric acid and 15% papain; group 4 – treated with 37% phosphoric acid and 6% bromelain; group 5 – treated with 37% phosphoric acid and 10% bromelain.

Tukey's test was used to compare the mean SBS values among all experimental groups (Table 2). The results showed statistically significant differences between the mean SBS values of group 2 (37% phosphoric acid + 10% papain) and group 3 (37% phosphoric acid + 15% papain), as well as between the mean SBS values of group 2 (37% phosphoric acid + 10% papain) and group 4 (37% phosphoric acid + 6% bromelain), with *p*-values of 0.004 and 0.017, respectively. The differences between the mean SBS values of group 4 (37% phosphoric acid + 6% bromelain)

Table 1. Shear bond strength values of the study groups

Study group	Shear bond strength [MPa]		
	M ±*SD*	minimum	maximum
Group 1 (37% phosphoric acid)	8.919 ±2.01	4.91	10.80
Group 2 (37% phosphoric acid and 10% papain)	6.98 ±2.59	3.99	12.20
Group 3 (37% phosphoric acid and 15% papain)	10.90 ±3.44	7.20	16.40
Group 4 (37% phosphoric acid and 6% bromelain)	10.83 ±4.40	6.28	16.50
Group 5 (37% phosphoric acid and 10% bromelain)	6.77 ±0.54	6.04	7.47

M – mean; *SD* – standard deviation.

Table 2. Pairwise comparison of mean shear bond strength values between all groups using Tukey's test

Study group	Group 1	Group 2	Group 3	Group 4	Group 5
Group 1 (37% phosphoric acid)	–	0.063	0.309	0.606	0.072
Group 2 (37% phosphoric acid and 10% papain)	0.063	–	0.004*	0.017*	0.991
Group 3 (37% phosphoric acid and 15% papain)	0.309	0.004*	–	0.616	0.005*
Group 4 (37% phosphoric acid and 6% bromelain)	0.606	0.017*	0.616	–	0.021*
Group 5 (37% phosphoric acid and 10% bromelain)	0.072	0.991	0.005*	0.021*	–

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

and group 5 (37% phosphoric acid + 10% bromelain), and between the mean SBS values of group 3 (37% phosphoric acid + 15% papain) and group 5 (37% phosphoric acid + 10% bromelain), were also statistically significant, with *p*-values of 0.021 and 0.005, respectively. However, no statistically significant differences were found between group 1 (control) and the other experimental groups (all *p*-values > 0.05).

Figure 4 presents the SEM images of the dentin surfaces in the various experimental groups, displaying the surface topography of the dentin substrate after different treatments. There was no collagen network covering the peritubular dentin in group 2 (37% phosphoric acid + 10% papain), group 3 (37% phosphoric acid + 15% papain) or group 4 (37% phosphoric acid + 6% bromelain). The orifices of the dentinal tubules in these groups were wider than those in the control group.

Discussion

The present study found that the application of bromelain and papain to dentin did not result in a statistically significant increase in SBS values. This observation contradicts the results of some previous studies.[11,16] Chauhan et al. reported that the deproteinization of dentin and removal of unsupported collagen fibers with bromelain treatment after acid etching could statistically improve the SBS of the adhesive system to dentin.[11] The application of the deproteinizing agent was reported to increase the permeability of the dentin substrate due to the reduction of collagen on the acid-etched surface and the widening of dentinal tubules on the outer surface of the exposed dentin.[11,17] Moreover, treatment with bromelain can increase the surface energy of dentin and the infiltration of monomers. Due to the high surface energy of hydroxyapatite and the low surface energy of collagen, the removal of the latter from etched dentin results in a reduction of organic content, an increase in surface energy and altered hydrophilic properties of the dentin, leading to better penetration of adhesive monomers.[18,19] In this study, group 3 (etched with 37% phosphoric acid and deproteinized with 15% papain) and group 4 (etched with 37% phosphoric acid and deproteinized with 6% bromelain) demonstrated higher SBS values compared to the other experimental groups. However, no significant difference in the SBS was observed between the control group and any of the groups deproteinized with bromelain or papain.

In agreement with the present findings, Hasija et al.[20] and Agarwal et al.[21] reported that the application of 10% papain after acid etching did not affect the SBS to enamel. The similarity between these results may be attributed to the use of papain and bromelain enzymes after the acid etching process. The results suggest that the use of deproteinization agents with lower acidity, such as papain or

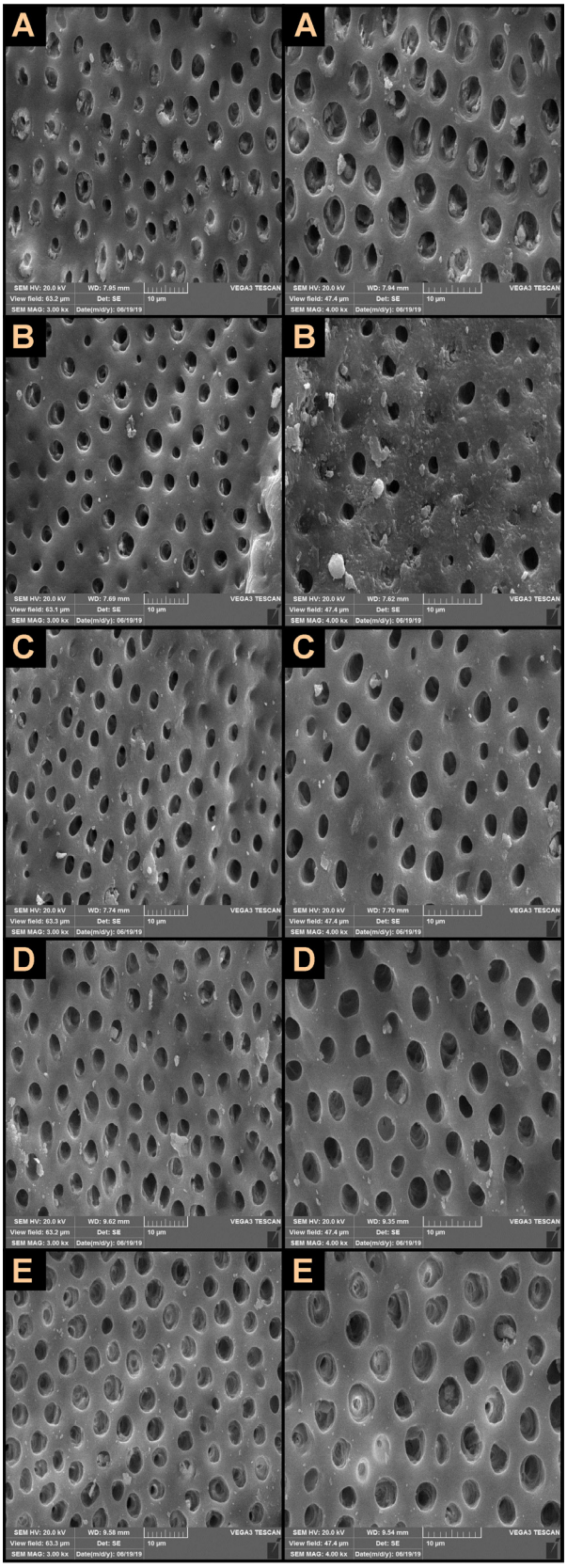

Fig. 4. Scanning electron micrographs of all groups taken at ×3,000 (left) and ×4,000 (right) magnification

A. Control surface etched with 37% phosphoric acid; B. Surface treatment with 37% phosphoric acid and 10% papain; C. Surface treatment with 37% phosphoric acid and 15% papain; D. Surface treatment with 37% phosphoric acid and 6% bromelain; E. Surface treatment with 37% phosphoric acid and 10% bromelain.

bromelain enzymes, after the acid etching process with phosphoric acid does not significantly increase the SBS. The present results are consistent with previous reports on the subject. However, it should be noted that the specimens in the current study were restored using a two-step, etch-and-rinse adhesive system and 1 type of composite resin. Other materials may perform differently, as observed in a previous investigation that used 10% papain before restoring enamel with RMGIC.[15]

The present study found that the group treated with 15% papain had a higher SBS than the group treated with 10% papain, which is consistent with a previous study.[14] The previous study demonstrated that enamel deproteinization with 8% or 10% papain gel increased the SBS of orthodontic brackets bonded with RMGIC, compared to deproteinization with lower concentrations of papain (2%, 4% and 6% gels).[14] However, in the present study, the mean SBS for the group deproteinized with 6% bromelain was higher than that of the group deproteinized with 10% bromelain. The decrease in bond strength that occurred following the application of the higher concentration of bromelain may be due to damage to the dentin organic matrix and collagen fibers at this concentration of the enzyme. Bromelain is a protease (proteolytic enzyme) that can catalyze the hydrolysis of dentin proteins and cleave their peptide chains. The increased proteolytic activity of bromelain at higher concentrations may negatively affect the mechanical properties of dentin by destroying its organic content.[22] Consequently, organic adhesive monomers may not be able to adequately infiltrate the demineralized dentin, resulting in reduced bond strength.

The SEM observations of the present study showed that there was no collagen network covering the peritubular dentin in the groups deproteinized with 10% papain, 15% papain and 6% bromelain. The orifices of the dentinal tubules appeared wider in these groups compared to the other groups. Our results are consistent with previous studies and may be explained by the depletion of collagen from the acid-etched dentin caused by the action of bromelain and papain.[16,23] However, the SEM observations in this study were inconsistent with the results of the SBS test, which showed that the same 3 groups – those deproteinized with 10% papain, 15% papain and 6% bromelain – had higher SBS values than the other groups, although no statistically significant difference was found between any of the treatment groups and the control group. This could be attributed to other factors, such as the type of adhesive system used. In agreement with the current findings, Kasraei et al. reported that the treatment of the acid-etched dentin surface with 5% bromelain before the application of the adhesive had no significant effect on marginal microleakage of Class V composite restorations.[23] The SEM micrographs of the resin–dentin interface after the application of bromelain showed that the hybrid layer and resin tags were thick, and the resin tags were conical in shape in the bromelain-treated group. Additionally, spherical residues were observed on resin tags due to the minor infiltration of resin into accessory canals in the dentin.[23]

In the present study, peritubular dentin and collagen fibers were observed in the control group, where the dentin surface had been treated with phosphoric acid. The possible explanation for this finding is that the action of phosphoric acid may be self-limited due to the buffering capacity of deep dentin.[24] Moreover, the average number of dentinal tubules is higher in the deep dentin than in the superficial dentin,[25] which suggests that the high surface moisture content of deep dentin might have affected the etching effectiveness of phosphoric acid during the removal of the smear layer.

The SEM micrographs obtained from the group deproteinized with 10% bromelain displayed similar characteristics to those of the control group. Some of the dentinal tubules were partially or completely obscured by smear plugs, and peritubular dentin was observed in both groups.

This study is the first to survey and evaluate the effects of different concentrations of bromelain and papain, 2 common proteolytic enzymes, on the SBS of composite resin to deep dentin using an etch-and-rinse adhesive system. However, there are some limitations to consider. This was an in vitro study and therefore could not precisely simulate oral conditions, such as water sorption, masticatory cycle, and pH and thermal changes. Therefore, some differences may be observed between the present results and clinical studies on vital teeth, which should be undertaken as a future extension of this work. Additional research with larger sample sizes and varying enzyme concentrations is necessary. It should be combined with other adhesive bonding systems, such as self-etch adhesive bonding systems, to develop the most appropriate method for increasing the bond strength of composite resin to deep dentin.

Conclusions

Deproteinization of deep dentin with various concentrations of papain and bromelain after acid etching did not significantly affect the SBS of composite resin to the treated dentin. However, the group deproteinized with 15% papain demonstrated a higher SBS, on average, than the group deproteinized with 10% papain. Similarly, the group deproteinized with 6% bromelain showed higher SBS values compared to the group deproteinized with 10% bromelain.

Ethics approval and consent to participate

The Ethics Committee of Shiraz University of Medical Sciences approved the research protocol (approval No. IR.SUMS.REC.1396.S509).

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

Adhesion of glass ionomer cements to primary dentin using a universal adhesive

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Abstract

Background. Glass ionomers are widely used for restoring carious primary teeth. However, their ability to bond to primary dentin is considered a challenge in pediatric dentistry.

Objectives. The study aimed to evaluate the microshear bond strength (μSBS) of a resin-modified glass ionomer (RMGI) and a high-viscosity glass ionomer cement (Hv-GIC) to primary dentin using a universal adhesive.

Material and methods. Thirty human primary maxillary canines were cut in half and prepared for the μSBS test. The specimens ($N = 60$) were assigned to 6 groups. Three groups were defined for RMGI (FUJI II LC) and 3 groups for Hv-GIC (EQUIA Forte): with an immediately curing adhesive (G-Premio); with a delayed curing adhesive; and without an adhesive (control group). After preparing the dentin surfaces, the glass ionomers were bonded using Tygon® tubes with an internal diameter of 0.7 mm. The μSBS test was performed, and the data was analyzed using two-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Additionally, the failure modes were determined using a stereomicroscope. Six specimens, one for each study group, were prepared for scanning electron microscopy (SEM) analysis to observe the glass ionomer–dentin interface.

Results. The type of glass ionomer did not have a significant effect on the μSBS ($p = 0.305$). Groups that received universal adhesive application prior to glass ionomer exhibited a significantly higher μSBS ($p < 0.0001$). However, there was no significant difference between the immediately curing and delayed curing groups ($p = 0.157$). The predominant failure mode was mixed failure.

Conclusions. Higher bond strength of glass ionomers to primary teeth can be achieved by using universal adhesives, which, in addition to the proven benefits of glass ionomers, can improve their clinical success.

Keywords: primary teeth, glass ionomer cements, adhesives

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Introduction

Glass ionomer cements (GICs) have been widely used in dentistry since their introduction.[1] Some properties such as fluoride release,[2] preservation of intact dental tissues and chemical adhesion to the tooth structure make them a desirable choice for restoring carious teeth.[3] The adhesion mechanism of GICs consists of hydrogen bonds promoted by their free hydrophilic carboxylic groups and a simultaneous ionic exchange at the interface.[4] Early conventional glass ionomers had some disadvantages, such as unfavorable strength and toughness.[5] To improve their physical properties, resin-modified glass ionomers (RMGIs) were developed by adding hydrophilic monomers.[6] In recent years, high-viscosity glass ionomer cements (Hv-GICs) have been developed by modifying the powder/liquid ratio and particle size.[7] This has resulted in improved fracture toughness and flexural strength, as well as lowered sensitivity to moisture compared to conventional GICs.[8]

Although composite resins usually have better esthetic and mechanical characteristics, their technique sensitivity can be a challenge, especially in less cooperative patients, such as children.[9] Therefore, in some cases, GIC is the preferred material for restoring carious primary teeth.[10] Additionally, the bond strength of restorative materials may be affected by a lower degree of mineralization and a higher tubular density in primary dentin compared to permanent dentin,[11,12] making the bond strength of materials to primary dentin a significant concern.

In recent years, universal adhesives (also known as multi-mode adhesives) have been introduced. These are single-bottle, no-mix adhesive systems that can provide adhesion to various substrates.[13,14] New-generation adhesives can be used in both self-etch and etch-and-rinse modes. However, the self-etch technique simplifies the application process and minimizes errors.[15]

Thus, the purpose of this in vitro study was to evaluate the bonding properties of an RMGI (FUJI II LC®; GC Corporation, Tokyo, Japan) and a Hv-GIC (EQUIA Forte™; GC Corporation) to the dentin of primary teeth. Since previous studies have shown the effect of bonding agents on improving the bond strength of RMGIs to permanent dentin,[16,17] we assessed the effect of using a universal adhesive (G-Premio BOND; GC Corporation) on the bond strength of these GICs to primary dentin.

Material and methods

This in vitro study was conducted in the Department of Pediatric Dentistry, in collaboration with the Department of Restorative Dentistry at Shahid Beheshti School of Dentistry, Tehran, Iran, after receiving approval from the Committee for Ethics in Research (No. IR.SBMU.DRC.REC.1398.011).

Thirty-six extracted human primary maxillary canines were selected, 6 of which were observed using field emission scanning electron microscopy (FE-SEM). The teeth were examined under a stereomicroscope (SZX9; Olympus, Tokyo, Japan) to confirm the absence of cracks, fractures, caries, restorations, hypoplasia, or anatomical abnormalities.

The teeth had been extracted for orthodontic reasons during the previous 3 months and were kept at room temperature in normal saline, which was replaced weekly. Crowns of the teeth were debrided using a prophylaxis brush on a low-speed handpiece for 30 s and disinfected with 0.5% chloramine T solution. Sixty specimens (N = 60) were obtained by sectioning 30 teeth into equal mesial and distal halves using a low-speed cutting machine (IsoMet® Low Speed Precision Cutter; Buehler, Lake Bluff, USA). The roots of all specimens were cut 2 mm below the cementoenamel junction and discarded. The dentin surfaces were polished for 10 s under running water using 400-, 600-, 800-, and 1000-grit silicon carbide grinding papers (Matador; STARCKE® GmbH & Co. KG, Melle, Germany), respectively.

We randomly assigned 60 specimens to 6 groups, applying EQUIA Forte in 3 groups with different surface treatment methods: with an immediately curing adhesive; with a delayed curing adhesive; and without an adhesive as control. The remaining 3 groups were treated with FUJI II LC using the same methods. Table 1 provides a summary of the dental materials and their composition. In the groups that used an immediately curing bonding agent, a universal adhesive (G-Premio) was applied to the dentin. After 10 s, the adhesive was dried with air spray for 5 s and then light-cured for 10 s using a light-emitting diode (LED) unit (Guilin Woodpecker Medical Instrument Co. Ltd., Guilin, China) with an intensity of 1000 mW/cm^2. In the delayed curing groups, the bonding agent was applied in the same manner; however, its light activation was delayed until the application of the glass ionomer.

Table 1. Dental materials and their composition

Name	Material type	Composition	Manufacturer
EQUIA Forte	high-viscosity glass ionomer cement	fluoroaluminosilicate glass, water, polyacrylic acid, polybasic carboxylic acid, camphorquinone	GC Corporation, Tokyo, Japan
FUJI II LC	resin-modified glass ionomer	powder: fluoroaluminosilicate glass liquid: water, polyacrylic acid, HEMA, urethane dimethacrylate	GC Corporation, Tokyo, Japan
G-Premio BOND	universal adhesive (8th generation)	10-MDP, 4-MET, MEPS, BHT, acetone, dimethacrylate resins, initiators, filler, water	GC Corporation, Tokyo, Japan

HEMA – hydroxyethyl methacrylate; 10-MDP – 10-methacryloyloxydecyl dihydrogen phosphate; 4-MET – 4-methacryloyloxyethyl trimellitate; MEPS – methacryloyloxyalkyl thiophosphate methylmethacrylate; BHT – butylated hydroxytoluene.

TYGON® tubes (Saint-Gobain, Paris, France) with an inner diameter of 0.7 mm and a height of 1 mm were prepared for packing the glass ionomers over the dentin samples. The powder and liquid in the FUJI II LC groups were mixed according to the manufacturer's instructions. The mixture was then packed into TYGON tubes placed over the dentin samples and light-cured for 20 s. In the EQUIA Forte groups, pre-loaded capsules were mixed for 10 s and packed into TYGON tubes placed over the samples. The samples were left to self-cure for 2 min and then covered with an EQUIA coat, a special coating that was light-cured for 20 s according to the manufacturer's instructions. In the delayed curing groups, light activation of the bonding agent and glass ionomer occurred simultaneously at this stage. After 24 h of immersion in distilled water and incubation at 37°C, the TYGON tubes were carefully removed using a scalpel. The specimens were then subjected to the microshear bond strength (μSBS) test.

Bond strength was measured using a Microtensile Tester (Bisco Inc., Schaumburg, USA) that was transformed into a microshear tester by attaching metallic cylinders with a diameter of 1 mm to one of its working plates. Samples were fixed onto the other working plate of the tester machine using a cyanoacrylate adhesive. An orthodontic wire (0.2 mm in diameter) was formed into a loop to connect the metallic cylinder to the base of the glass ionomer cylinder. The bonded interface, the wire loop and the center of the metallic cylinder were aligned as straight as possible. Microshear forces were applied at a crosshead speed of 0.5 mm/min until debonding occurred, and the μSBS was recorded.

Failure modes were evaluated using the stereomicroscope at ×20 magnification. The results were recorded as adhesive (fracture at the glass ionomer–dentin interface), cohesive (fracture within the glass ionomer or bonding agent) or mixed (a combination of both failures).

The remaining 6 primary canines, one for each study group, were selected for SEM observations. We cut the upper third of the crowns to expose a flat dentin surface. We prepared the dentin surfaces in the same way as the previous specimens and applied a bulk of glass ionomer. Then, we longitudinally sectioned the specimens using an IsoMet Low-Speed Precision Cutter to reach the glass ionomer–dentin interface. The specimens were polished using silicon carbide papers with grits of 400, 600, 800, 1000, and 2000, followed by cleaning with 37% phosphoric acid for 5 s and thorough rinsing for 30 s. All specimens were dehydrated using a desiccator containing silica gel for 24 h. After sputter coating, the glass ionomer–dentin interfaces were observed using a FE-SEM (S-4160; Hitachi, Tokyo, Japan) at ×500 magnification.

Statistical analysis

Data was analyzed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). A two-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was used to determine the difference in bond strength between the groups. Failure mode analysis was conducted using the χ^2 tests. The level of significance set for the study was ≤0.05.

Results

Descriptive statistics of μSBS for the 6 groups are shown in Table 2. According to the results of the two-way ANOVA test, the glass ionomer type did not have a significant effect on μSBS (p = 0.305). However, the effect of surface treatment was significant (p < 0.0001). In addition, the interaction effect between surface treatment and the type of glass ionomer was not significant (p = 0.558). Post hoc Tukey's Honest Significant Difference (HSD) test was performed to compare 3 surface treatment methods (i.e., an immediately curing adhesive, a delayed curing adhesive, and without an adhesive). It was shown that the groups with an adhesive had significantly higher μSBS values than the control group (p < 0.0001). However, there was no significant difference between the immediately curing and delayed curing adhesive groups (p = 0.157).

The distribution of failure modes among the 6 study groups did not show any significant difference (p = 0.974). The most frequently observed failure mode was mixed failure, while the cohesive mode had the lowest proportion. Table 2 shows the distribution of failure modes within the groups.

Table 2. Descriptive statistics of microshear bond strength (μSBS) and failure mode for the 6 groups

Group	μSBS [MPa] *M* ±*SD*	Failure mode [%]		
		adhesive	cohesive	mixed
FUJI II LC without an adhesive	6.72 ±1.28	40	10	50
FUJI II LC with an immediately curing adhesive	10.72 ±1.61	40	20	40
FUJI II LC with a delayed curing adhesive	9.58 ±0.96	30	20	50
EQUIA Forte without an adhesive	6.3 ±1.1	50	0	50
EQUIA Forte with an immediately curing adhesive	10.02 ±1.24	40	10	50
EQUIA Forte with a delayed curing adhesive	9.71 ±1.06	40	20	40

M – mean; *SD* – standard deviation.

The SEM images of the glass ionomer–dentin interfaces are presented in Fig. 1. All groups showed gap formation. In the groups with an immediately curing adhesive, the fracture occurred within the glass ionomer, and the glass ionomer–adhesive interaction was maintained in most parts. However, in the groups with a delayed curing adhesive, the layer of bonding agent was imperceptible.

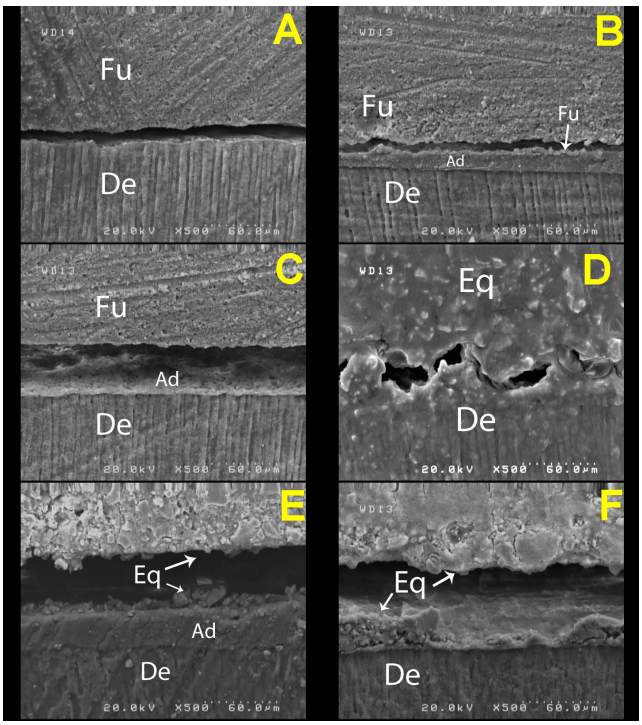

Fig. 1. Scanning electron microscopy (SEM) images (×500 magnification)
A. FUJI II LC without an adhesive; B. FUJI II LC with an immediately curing adhesive; C. FUJI II LC with a delayed curing adhesive; D. EQUIA Forte without an adhesive; E. EQUIA Forte with an immediately curing adhesive; F. EQUIA Forte with a delayed curing adhesive. Fu – FUJI II LC; De – dentin; Ad – adhesive; Eq – EQUIA Forte.

Discussion

The bond strength of restorative dental materials plays a crucial role in the treatment success by preventing marginal gapping and microleakage.[4,18] We aimed to assess the bonding properties of RMGI (FUJI II LC) and Hv-GIC (EQUIA Forte) to primary dentin, considering the limited evidence on the bond strength of GICs to primary teeth and the improved physical characteristics of modified glass ionomers. FUJI II LC is a commonly used material in pediatric dentistry, while EQUIA Forte is a new-generation glass ionomer. Previous studies have not evaluated their bonding properties in primary teeth.

Both macro- and micro-tests can be used to measure bond strength, either tensile or shear. However, they differ in the cross-sectional area being bonded. This study used a micro-test to minimize false failures and errors that may occur due to the larger size of samples in macro-tests.[19] The μSBS measurement method is useful for materials like glass ionomers, because their properties make them susceptible to the specimen preparation and testing conditions of micro-tensile bond strength tests.[20] In our laboratory, we converted the design of a micro-tensile testing machine into a microshear tester, as described in the methods section. Shear force was applied using a wire loop, as described in previous studies.[21–23] The wire loop design allows for stress to be concentrated closer to the interface area compared to the the knife-edge design.[19]

When evaluating the effect of bonding agent application prior to glass ionomers, we observed a significantly improved bond strength in both types of glass ionomers. Since RMGIs contain resin components, the benefits of both a chemical bond through ionic exchange and a micromechanical bond can be achieved by using a bonding agent.[24] This observation has been reported in previous studies that used various types of bonding agents. Nakanuma et al.[16] reported a significant improvement in the tensile bond strength of FUJI II LC to permanent dentin when using a dentin bonding agent in addition to different primers. Similarly, Poggio et al.[25] found that the bond strength of FUJI II LC to bovine dentin significantly increased when using a self-etch adhesive prior to the glass ionomer, which is consistent with our results. Besnault et al.[26] observed a significantly higher shear bond strength of FUJI II LC to permanent dentin using 7 different self-etch adhesives, which is consistent with our study. The bond strength of FUJI II LC increased after the application of a universal adhesive in our study, which may be due to several factors, including the presence of unsaturated carbon–carbon bonds in the FUJI II LC and G-Premio bonds, which can form covalent bonds during polymerization. Additionally, the hydrophilic nature of both RMGI and a universal adhesive can improve the compatibility between the 2 materials.[26]

The evidence regarding the effect of bonding agents on the bond strength of EQUIA Forte is limited. However, it is presumed that a chemical reaction occurs between the universal adhesive and the calcium ions of EQUIA Forte via dihydrogen phosphate groups of its 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP),[27] which can explain the bond strength improvement observed in our study.

According to our results, the bond strength measurements were not significantly affected by the type of glass ionomer. This is consistent with the results of a previous study by Latta et al.,[28] which compared the shear bond strength of FUJI II LC and EQUIA Forte on permanent teeth and found no significant differences between them. Yao et al.[29] showed a significantly higher tensile bond strength of FUJI II LC to both flat and cavity-formed permanent dentin when compared to EQUIA Forte. This discrepancy might be justified by the fact that no dentin pre-treatments were applied in the EQUIA Forte group in their study, which could have reduced micromechanical interlocking formation due to the interference of the

smear layer. The polished surfaces in our specimens minimize the smear layer thickness and provide better bonding opportunities for self-adhesive materials. Additionally, the abovementioned study did not apply an EQUIA Forte coat. This resin-based coating agent fills the porosities and cracks and prevents the early setting of GICs in a moist environment.[29]

The most frequently observed failure mode in both GICs was mixed failure, which is consistent with a study by Abdelmegid et al.[30] In SEM observations, the interactions between the glass ionomer and adhesive were maintained in all specimens. However, gap formation was evident after desiccation and resin tags were not observed. In a previous study conducted by Pereira et al.,[17] resin tags were formed in FUJI II LC. This difference could be attributed to the surface treatment methods and the type of adhesive system used.

A meta-analysis has shown that GICs have clinical performance comparable to composite resins in various aspects, including marginal adaptation and secondary caries, for Class II restoration of primary teeth.[9] Therefore, GICs can be considered for permanent restoration of primary teeth, especially in high-caries patients.[3] A universal adhesive can increase the bond strength of GICs and contribute to their improved clinical performance.

One of the limitations of the present study was its in vitro design, which overlooks the impact of the oral environment on bond durability. Since the measurements were performed on healthy dentin, further studies on caries-affected dentin and long-term evaluations of bond strength are recommended.

Conclusions

The bond strength of the examined glass ionomers was significantly improved by applying a universal adhesive to primary dentin. Our results suggest that this improvement can increase the clinical success of glass ionomer restorations in primary teeth, given the desirable properties of GICs.

Ethics approval and consent to participate

The study was approved by the Committee for Ethics in Research (approval No. IR.SBMU.DRC.REC.1398.011).

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

Root coverage with the restoration of non-carious cervical lesions: A systematic review and meta-analysis

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Abstract

The progression of non-carious cervical lesions (NCCLs) leads to gingival recession (GR), which is restored with restorative materials, using different periodontal plastic surgery procedures. There is no consensus on which technique is superior to others. Therefore, the present systematic review aimed to assess the effectiveness of root coverage (RC) procedures in the restored and unrestored NCCLs in terms of clinical and patient-centered outcomes.

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) while searching 5 databases in addition to the gray literature. The Medical Subject Headings (MeSH) terms and keywords in the title and abstract fields, as well as in headings, were used to search the existing literature for the relevant publications on the effectiveness of RC procedures with the restoration of NCCLs over the past 3 decades (January 1990–July 2021). After applying the inclusion and exclusion criteria, 13 articles were read in full and critically analyzed. The quality analysis was performed using the Cochrane RevMan software.

A total of 222 potentially relevant titles and abstracts were found after the initial electronic and manual search, and after removing duplicates. Applying the inclusion and exclusion criteria yielded 23 publications that were further analyzed for relevance and applicability. Following critical analysis, 13 publications were used for validity assessment and data extraction.

In the teeth with NCCLs and GR, the restoration of NCCLs does not affect the percentage RC. However, it significantly decreases dentin hypersensitivity, and the patients' perception of esthetics and satisfaction.

Keywords: gingival recession, root coverage, non-carious cervical lesions, periodontal plastic surgery, mucogingival surgery

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Introduction

Due to the reduction of caries prevalence in worldwide populations, the teeth are functional for more extended periods.[1] This may expose the teeth to conditions other than caries, such as non-carious cervical lesions (NCCLs). Non-carious cervical lesions are saucer- or wedge-shaped defects present at the cementoenamel junction (CEJ), characterized by a gradual, slow loss of mineralized dental tissue in the absence of dental caries. The progression of NCCLs causes the loss of CEJ, leading to crown and root involvement.[2] Most of the NCCL coronal zone may be formed by the exposed dentin of the anatomical crown, and the apical zone involves the dentin of the anatomical root. Furthermore, the apical shift of the gingival margin with the exposure of the root surface leads to gingival recession (GR).[3] Non-carious cervical lesions are restored with various materials, like glass ionomer cement (GIC), resin-modified glass ionomer cement (RMGIC) or composites. These restorations reduce dentinal hypersensitivity, but do not provide root coverage (RC) and improve esthetics.[4]

The successful treatment of NCCLs associated with GR is based on clinically predictable periodontal plastic surgery procedures with the restoration of NCCLs. Periodontal plastic surgery procedures may comprise the coronally advanced/positioned flap or the connective tissue graft (CTG) over the restored root surfaces. Various studies have shown that GR associated with NCCLs can be successfully treated with a restorative procedure combined with a periodontal plastic surgery procedure to obtain optimal functional and esthetic results. The restoration of NCCLs followed by mucogingival surgery is indicated when cervical abrasion is associated with GR of more than 3 mm.[5–7]

The RC of the restored surfaces depends on the extent of NCCL and GR, the amount of interdental bone and soft tissue loss, the type of restoration used, and the periodontal procedure performed.[8]

Several RC procedures have been demonstrated to correct GR, but there is no consensus on which is superior. Regarding the restorations, various materials have been shown to effectively restore NCCLs, claiming superiority over one another. However, there is no consensus on which treatment is better at correcting this complex lesion of RC associated with GR. Therefore, the present systematic review was undertaken to assess the effectiveness of RC procedures in the restored and unrestored NCCLs in terms of clinical and patient-centered outcomes.

The focused question was: What is the success rate of root coverage procedures in patients with GR associated with NCCLs?

Material and methods

Report and protocol

This review was prepared in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement,[9] the Cochrane Collaboration guidelines, and used a review checklist based on the proposed focused question. It was registered with PROSPERO (https://www.crd.york.ac.uk/PROSPERO) under the registration number CRD42021258035.

Inclusion and exclusion criteria

The inclusion criteria for this systematic review were based on the PICOS acronym:

– (P) types of participants: patients suffering from GR defects associated with NCCLs;
– (I) types of interventions: any type of NCCL restoration followed by any RC procedure;
– (C) comparisons between interventions: all possible comparisons among the groups, including the restored and unrestored NCCL with RC procedures;
– (O) type of outcome measures:
  • primary outcome: recession height (RH); keratinized tissue thickness (KTT); and keratinized tissue height (KTH);
  • secondary outcome: probing depth (PD); clinical attachment loss (CAL); plaque index (PI); and gingival index (GI);
– (S) types of studies: only randomized controlled trials (RCTs) with at least 6 months of follow-up and including at least 15 patients in each arm.

The exclusion criteria were as follows: non-randomized controlled trials; defects other than NCCLs; and less than 6 months of follow-up.

Search strategy

An initial search strategy with no restrictions regarding the status or publication language was performed to identify relevant studies published up to and including July 31, 2021, that met the inclusion criteria. The studies selected for the review were RCTs with at least a 6-month follow-up that utilized tooth-colored restorative materials for NCCLs and RC procedures.

The keywords used: 'non-carious cervical lesions'; 'cervical abrasion'; 'cervical restoration'; 'tooth-colored restorative materials'; 'root coverage'; 'gingival recession'; 'periodontal plastic surgery'; 'coronally advanced flap'; 'laterally displaced flap'; 'denuded root surface'; 'abfraction'; 'subepithelial connective tissue graft'; and 'mucogingival surgery'.

Electronic search

The MEDLINE (via PubMed) search strategy relied on the Cochrane Highly Sensitive Search Strategy for identifying randomized trials in MEDLINE: Sensitivity-maximizing version (2008 revision); PubMed format.[10] The following electronic databases were searched: MEDLINE via PubMed; Scopus; the Cochrane Central Register of Controlled Trials (CENTRAL); Embase; and the Web of Science.

Hand-searching and the gray literature

The following leading journals were hand-searched twice by the 2 review authors (KC and LG): "Journal of Periodontology"; "Journal of Clinical Periodontology"; "Journal of Periodontal Research"; "International Journal of Periodontics and Restorative Dentistry'; and "Journal of Indian Society of Periodontology".

The gray literature was explored using the Conference Proceedings Citation Index (CPCI) within the Web of Science, the System for Information on Grey Literature in Europe (SIGLE) database, and the Scopus Web and Patent results sets. Dissertations and theses were searched using the ProQuest Dissertations & Theses Global (PQDT)™ full-text database. To locate unpublished and ongoing trials related to the review question, the Current Controlled Trials (www.controlled-trials.com) and ClinicalTrials.gov (www.clinicaltrials.gov) trial registries were consulted.

The review authors checked twice the bibliographies of all the RCTs and relevant review articles included.

Each study identified by at least one review author through the various search strategies was involved in the next stage (study selection).

Study selection

The titles and abstracts (when available) of all reports (222 articles) identified through the electronic and manual search were screened independently by the 2 review authors. The full texts were obtained if the studies appeared to meet the inclusion criteria or if there was insufficient data in the title and abstract to make a clear decision. The complete reports obtained from all electronic sources and with other searching methods were assessed independently by the 2 review authors to establish whether or not the studies met the inclusion criteria. The 2 review authors discussed any disagreement to resolve conflicts. Initially, 23 articles were selected after screening, and 10 not meeting the criteria were excluded, with the reasons for exclusion after the full-text analysis recorded.[11–20] Thirteen studies meeting the inclusion criteria were then included, and underwent validity assessment and data extraction. The screening and selection of articles are depicted in Fig. 1.

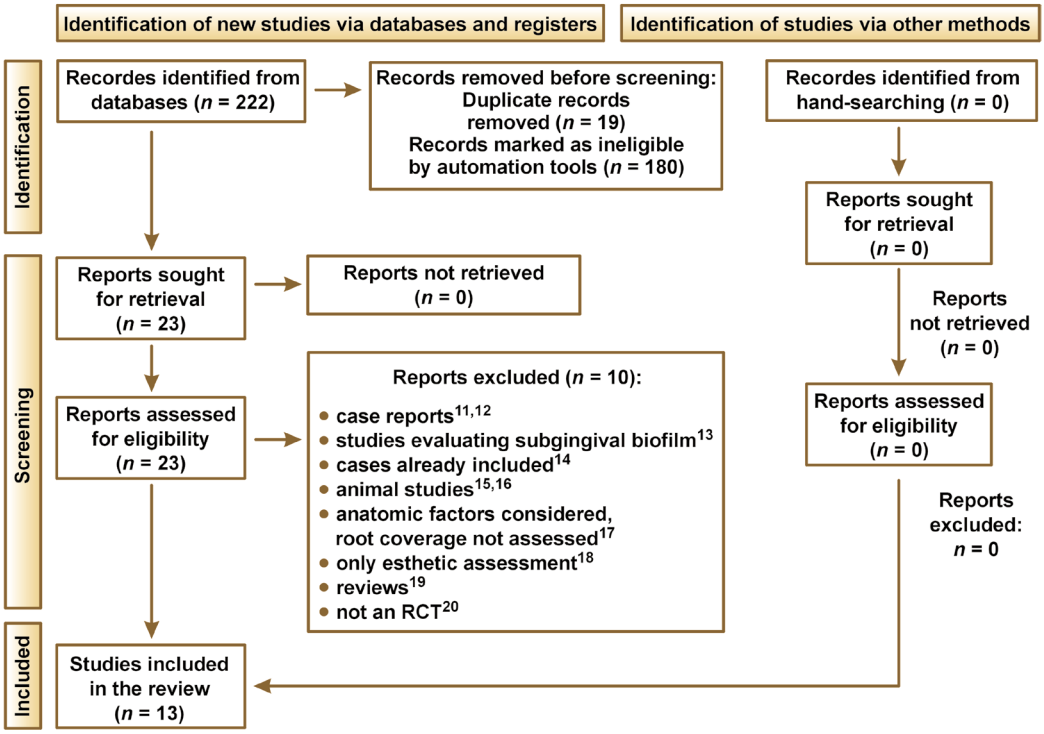

Fig. 1. Flow chart for the screening and selection of articles

RCT – randomized clinical trial.

Data extraction

The 2 review authors extracted data from the included studies independently, using the preferred data extraction forms. If necessary, trial authors were contacted for clarification or the missing information. For each trial included in the study, the following data was recorded:

– general information: year of publication; correspondence details; country of origin; and setting (university or clinical);
– methods: study design; and number of centers;
– participants: number of participants and their characteristics (age, gender and systemic health); and criteria for inclusion and exclusion;
– interventions and comparisons: number of intervention groups; types of intervention (restored or unrestored NCCLs); and surgical technique (type of RC procedure used – coronally advanced flap (CAF), CTG, or a combination);
– outcomes: details of the outcomes collected (types – RH, KTT, KTH, PD, CAL, PI, GI, time points, and patient-centered outcomes);
– results: number of participants allocated to each intervention group; dropouts; characteristics of patients in each group (age, gender and systemic health); and results for each outcome considered;
– study funding: information about the possible study funding.

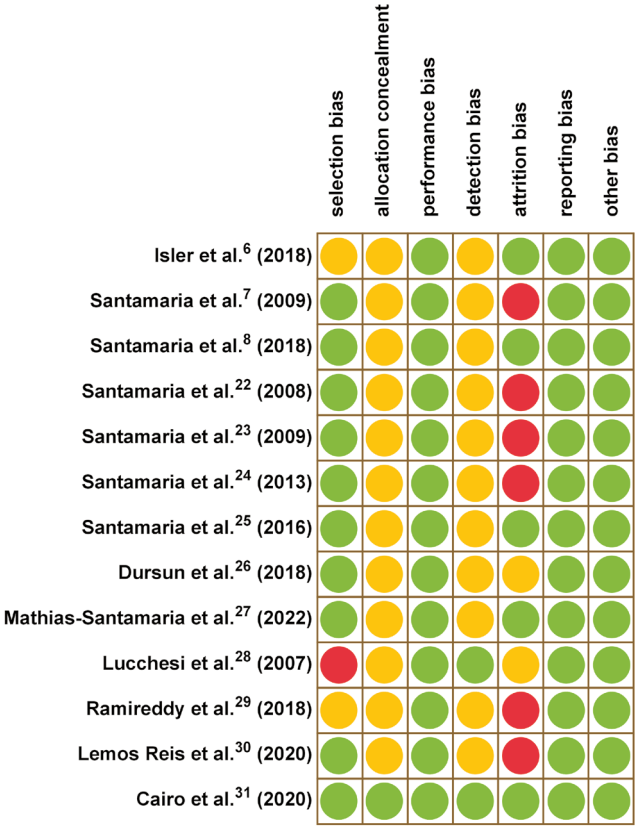

Fig. 2. Risk of bias assessment

Colors: green –low risk of bias; yellow – unclear risk of bias; red – high risk of bias.

Risk of bias assessment

The assessment of the risk of bias was carried out independently and in duplicate by the 2 review authors as part of the data extraction process, with any disagreement discussed between the same 2 review authors. It was conducted using the recommended approach for assessing the risk of bias in studies (Fig. 2) by the Cochrane Collaboration[21] and reported using the RevMan software, v. 5 (Copenhagen, Denmark: The Nordic Cochrane Centre, the Cochrane Collaboration).

Randomization

All trials were reported as RCTs, but not all of them reported randomization and allocation in detail.

Allocation

All trials presented an adequate method of allocation concealment except 2 studies.[6,22]

Masking

Examiner masking was not practical in most of the studies,[6,7,23–26] as it was easy to note whether the restoration was present. The investigators who recruited patients and the examiner were masked to the procedure in the study by Mathias-Santamaria et al.[27]

Data synthesis and reporting

This systematic review included 13 research articles that were analyzed for quantitative data. The literature analysis revealed that most studies were conducted from 2007 until now. Table 1 demonstrates the baseline characteristics of the RCTs included.

Table 2 reports the outcomes of the studies on which the meta-analysis was performed.

Statistical analysis

Data was extracted and coded using Microsoft Excel (Microsoft Corporation, Redmond, USA). The outcomes of interest were the mean RC, PD, KTH, keratinized tissue width (KTW), and CAL. The effect sizes and weights were calculated for each outcome from each study. The χ^2-based test of homogeneity was performed using Cochran's Q statistic. The I^2 statistic (<50%) indicated that there was homogeneity between the studies, hence a fixed effects model was considered. Forest plots were constructed for each outcome.

Table 1. Baseline characteristics of the included studies

Study	Year	Country	Setting	No. of centers	Study design	No. of participants	Age group [years]	External funding	Type of defect	Intervention		Primary and secondary outcomes	Follow-up
										test	control		
Isler et al.[6]	2018	Turkey	university	1	RCT	23	28–59	–	GR associated with NCCL	NRC +CTG RMGIC + CTG giomer + CTG	CTG	rRH, KTT, KTW, PD, rCAL	1 year
Santamaria et al.[7]	2009	Brazil	university/ college	1	RCT (split-mouth study – bilateral defects)	16	26–58	–	Miller's class I buccal GR associated with NCCL	R +CAF	CAF	KTT, KTW, PD, rCAL, BOP, RGR, CLH, DS	2 years
Santamaria et al.[8]	2018	Brazil	university/ college	1	RCT	40	22–60	–	Miller's class I or class II GR associated with NCCL (B+ tooth cervical defect)	PR +CTG	CTG + odontoplasty	KTT, KTW, PD, rCAL, PI, BOP, FMBI, RGR, CDH, CDC, RC	1 year
Santamaria et al.[22]	2008	Brazil	university/ college	1	RCT (split mouth study – bilateral defects)	19	24–58	–	Miller's class I buccal GR associated with NCCL	R + CAF	CAF	KTT, KTW, PD, BOP, RGR, CAL, CLH, DS	45 days, 2, 3, 6 months
Santamaria et al.[23]	2009	Brazil	university/ college	1	RCT	40	19–71	–	Miller's class I buccal GR associated with NCCL	R + CTG	CTG	KTT, KTW, PD, CAL, FMPI, BOP, FMBI, RGR, CLH	6 months
Santamaria et al.[24]	2013	Brazil	university/ college	1	RCT	36	19–71	–	Miller's class I buccal GR associated with NCCL	R + CTG	CTG	PD, CAL, BOP, RGR, CLH	2 years
Santamaria et al.[25]	2016	Brazil	university/ college	1	single-blind, parallel, equivalence RCT	36	–	–	Miller's class I or class II GR associated with NCCL (B+ tooth cervical defect)	RCom + CTG	CTG	KTT, KTW, PD, rCAL, FMPI, FMBI, RGR, CLH, DS	6 months, 1 year
Dursun et al.[26]	2018	Turkey	university	1	–	36	41.65 ±12.26 (*M* ±*SD*)	–	GR associated with NCCL	RMGIC + SCTG NIC + SCTG	–	KTH, WGR, HGR, CDH, DS	1 year
Mathias-Santamaria et al.[27]	2022	Brazil	university	1	double-blind, parallel RCT	62	>18	the State of São Paulo Research Foundation (FAPESP), Brazil (grants No. 2018/03284-3 and 2016/26154-2)	GR type 1 associated with class B+ or B– NCCL	CAF + CM	CAF	primary: CDC, RC secondary: KTT, KTW, PD, CAL, FMPI, FMGI, RGR, CDH, DH, mRES, VAS	baseline, 6 months, 1 year

Study	Year	Country	Setting	No. of centers	Study design	No. of participants	Age group [years]	External funding	Type of defect	Intervention		Primary and secondary outcomes	Follow-up
										test	control		
Lucchesi et al.[28]	2007	Brazil	university	1	–	59	23–65	–	root exposure with NCCL, root exposure without NCCL	test I: RMGIC + CPF test II: MRC + CPF (root exposure with NCCL)	CPF (root exposure without NCCL)	KTT, KTW, PD, PI, CALG, BOP, RR	6 months
Ramireddy et al.[29]	2018	India	university/ college	1	RCT	20 participants with 78 sites	24–58	–	Miller's class I or class II GR (single and multiple)	RMGIC + CAF	CAF + PRF	KTT, KTW, PD, rCAL, CLH, CLW, rGR, DS	6 months
Lemos Reis et al.[30]	2020	Brazil	university	1	controlled clinical trial (split-mouth study – bilateral defects)	17	24–65	the study was financially supported by the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil; the acellular dermal matrix was donated by BioHorizons Implant Systems, Inc., Birmingham, USA	test group – GR + NCCL control group – GR	CAF + ADMG	CAF + ADMG	KTT, KTW, PD, CAL, GR, RC	6 months
Cairo et al.[31]	2020	Italy	university	1	RCT	24 participants (12 – test group, 12 – control group)	>18	–	presence of NCCL associated with GR	CAF + CTG	CAF	KT, PD, CAL, GT, GR, IM–CEJr, IM–GM, IM–GM1, IM–MGJ, CEJr-BC, CRC, sens VAS, RES intra-operatory measurement	3, 6 months, 1 year

M – mean; *SD* – standard deviation; GR – gingival recession; NCCL – non-carious cervical lesion; NRC – nanofilled resin composite; CTG – connective tissue graft; RMGIC – resin-modified glass ionomer cement; R – restoration; CAF – coronally advanced flap; PR – partial restoration; RCom – resin composite; SCTG – subepithelial connective tissue graft; NIC – nano-ionomer cement; CM – collagen membrane; CPF – coronally positioned flap; MRC – microfilled resin composite; ADMG – acellular dermal matrix graft; PRF – platelet-rich fibrin; (r)RH – (relative) recession height; KTT – keratinized tissue thickness; KTH – keratinized tissue height; PD – probing depth (PD); CAL – clinical attachment loss; BOP – bleeding on probing; RGR – relative gingival recession; CLH – cervical lesion height; DS – dentin sensitivity; PI – plaque index; FMBI – full-mouth bleeding index; CDH – combined defect height; CDC – combined defect coverage; RC – root coverage; FMPI – full-mouth plaque index; KTH – keratinized tissue height; WGR – width of gingival recession; HGR – height of gingival recession; FMGI – full-mouth gingival index; mRES – modified root coverage esthetic score; VAS – visual analog scale; CALG – clinical attachment level gain; RR – recession reduction; CLW – cervical lesion width; KT – keratinized tissue; GT – gingival thickness; IM–CEJr – distance from the incisal margin (IM) to the restored cementoenamel junction (CEJ) level; IM–GM – distance from the gingival margin (GM) to IM; IM–GM1 – distance from GM to IM after suturing; IM–MGJ – distance from IM to the mucogingival junction (MGJ); CEJr–BC – distance from the restored CEJ to the bone crest after flap elevation; CRC – complete root coverage; RES – root coverage esthetic score.

Table 2. Outcomes of the studies included in the meta-analysis

Study	Year	Fully restored/ partially restored	Restoration used	Intervention	Follow-up	Outcome assessed													
						PI [%]	GI/ BOP [%]	PD [mm]	rCAL/ CAL [mm]	CALG [mm]	rRH/ RGR [mm]	RGR reduction [mm]	KTH / KTW	KTT [mm]	RR	RC [%]	CLH [mm]	CLH [%]	CDC [%]
Lucchesi et al.[28]	2007	fully restored	RMGIC + MRC	root exposure with NCCL treated with RMGI + CPF	baseline	–	–	1.5 ±0.5	–	–	–	–	3.58 ±0.94	0.83 ±0.37	–	–	–	–	–
					3 months	–	–	1.4 ±0.5	2.2 ±1.0	–	–	–	3.55 ±0.94	0.90 ±0.38	2.4 ±1.0	–	–	–	–
					6 months	–	–	1.4 ±0.5	2.2 ±1.0	–	–	–	3.70 ±0.98	0.93 ±0.37	2.4 ±1.0	–	–	–	–
				root exposure with NCCL treated with MRC + CPF	baseline	–	–	1.3 ±0.5	–	–	–	–	3.37 ±1.01	0.92 ±0.25	–	–	–	–	–
					3 months	–	–	1.4 ±0.8	2.3 ±0.8	–	–	–	3.05 ±0.71	0.92 ±0.25	2.3 ±0.7	–	–	–	–
					6 months	–	–	1.3 ±0.6	2.2 ±0.7	–	–	–	3.32 ±0.82	0.92 ±0.25	2.2 ±0.6	–	–	–	–
				root exposure without NCCL treated with CPF	baseline	–	–	1.4 ±0.5	–	–	–	–	3.81 ±0.91	0.9 3±0.34	–	–	–	–	–
					3 months	–	–	1.3 ±0.6	2.4 ±1.0	–	–	–	3.05 ±0.76	0.90 ±0.31	2.2 ±0.7	–	–	–	–
					6 months	–	–	1.2 ±0.5	2.4 ±1.0	–	–	–	3.40 ±0.94	0.88 ±0.28	2.2 ±0.7	–	–	–	–
Santamaria et al.[22]	2008	fully restored	RMGIC	R + CAF	baseline	–	–	1.18 ±0.40	12.12 ±1.13	–	10.94 ±1.32	–	–	–	–	–	2.63 ±0.57	–	–
					45 days	–	–	1.12 ±0.34	10.66 ±1.19†	–	9.54 ±1.21†	–	–	–	–	–	–	–	–
					2 months	–	–	1.06 ±0.44	10.57 ±1.21†	–	9.51 ±1.23†	–	–	–	–	–	–	–	–
					3 months	–	–	1.00 ±0.36	10.54 ±1.22†	–	9.54 ±1.25†	–	–	–	–	–	–	–	–
					6 months	–	–	1.00 ±0.36	10.54 ±1.20†	1.52 ±0.66	9.54 ±1.25†	1.39 ±0.29	–	–	–	–	–	56.14 ±11.74	–
				CAF	baseline	–	–	1.25 ±0.44	11.82 ±0.80	–	10.57 ±0.94	–	–	–	–	–	2.60 ±0.39	–	–
					45 days	–	–	1.50 ±0.51	10.43 ±0.90†	–	8.93 ±1.00†	–	–	–	–	–	–	–	–
					2 months	–	–	1.43 ±0.51	10.42 ±1.17†	–	8.99 ±0.89†	–	–	–	–	–	–	–	–
					3 months	–	–	1.37 ±0.50	10.40 ±1.10†	–	9.03 ±0.87†	–	–	–	–	–	–	–	–
					6 months	–	–	1.31 ±0.47	10.32 ±1.03†	1.50 ±0.73	9.01 ±0.83†	1.50 ±0.30	–	–	–	–	–	59.78 ±11.11	–

Study	Year	Fully restored/ partially restored	Restoration used	Intervention	Follow-up	Outcome assessed													
						PI [%]	GI/ BOP [%]	PD [mm]	rCAL/ CAL [mm]	CALG [mm]	rRH/ RGR [mm]	RGR reduction [mm]	KTH / KTW	KTT [mm]	RR	RC [%]	CLH [mm]	CLH [%]	CDC [%]
Santamaria et al.[7]	2009	fully restored	RMGIC	R + CAF	baseline	–	–	1.25 ±0.44	11.73 ±1.15	–	10.48 ±1.09	–	3.16 ±0.85	1.16 ±0.13	–	–	–	–	–
					6 months	–	–	1.00 ±0.36	10.14 ±0.95*	–	9.14 ±1.00*	–	–	–	–	–	–	–	–
					1 year	–	–	1.12 ±0.50	10.30 ±1.26*	–	9.17 ±0.99*	–	–	–	–	–	–	–	–
					2 years	–	–	1.25 ±0.44	10.42 ±1.00*	–	9.17 ±1.00*	–	3.11 ±0.91	1.07 ±0.20	–	80.37 ±25.44	2.54 ±0.50	51.57 ±17.20	–
				CAF	baseline	–	–	1.31 ±0.47	11.56 ±0.72	–	10.25 ±0.81	–	3.24 ±0.40	1.12 ±0.16	–	–	–	–	–
					6 months	–	–	1.37 ±0.50	10.21 ±.83*	–	8.84 ±0.77*	–	–	–	–	–	–	–	–
					1 year	–	–	1.50 ±0.51	10.37 ± 0.95*	–	8.87 ±0.81*	–	–	–	–	–	–	–	–
					2 years	–	–	1.50 ±0.50	10.36 ±0.97*	–	8.86 ±0.80*	–	3.25 ±0.56	1.04 ±0.33	–	83.46 ±20.79	2.58 ±0.42	53.87 ±12.60	–
Santamaria et al.[23]	2009	fully restored	RMGIC	R + CTG	baseline	18.5	14.0	1.10 ±0.44	–	–	11.79 ±1.09	–	2.54 ±1.17	0.85 ±0.19	–	–	3.27 ±0.68	–	–
					45 days	–	–	1.90 ±0.64*	–	–	9.50 ±0.87	–	–	–	–	–	–	–	–
					2 months	–	–	2.00 ±0.56*	–	–	9.51 ±0.88	–	–	–	–	–	–	–	–
					3 months	–	–	2.00 ±0.56*	–	–	9.57 ±0.89	–	–	–	–	–	–	–	–
					6 months	–	–	2.15 ± 0.67*	–	1.26 ±0.90	9.48 ±0.82	2.31 ±0.74	3.34 ±0.91	1.95 ±0.42	–	88.64 ±11.90	–	70.00 ±13.85	–
				CTG	baseline	19.4	18.0	1.15 ±0.48	–	–	11.70 ±2.01	–	2.38 ±1.22	0.90 ±0.23	–	–	3.22 ±0.52	–	–
					45 days	–	–	1.98 ±0.60	–	–	9.12 ±1.55	–	–	–	–	–	–	–	–
					2 months	–	–	2.00 ±0.45	–	–	9.15 ±1.46	–	–	–	–	–	–	–	–
					3 months	–	–	2.15 ±0.48	–	–	9.12 ±1.52	–	–	–	–	–	–	–	–
					6 months	–	–	2.10 ±0.55	–	1.58 ±0.74	9.17 ±1.53	2.53 ±0.78	3.05 ±1.11	1.93 ±0.53	–	91.91 ±70.76	–	77.59 ±20.15	–

Study	Year	Fully restored/ partially restored	Restoration used	Intervention	Follow-up	Outcome assessed													
						PI [%]	GI/ BOP [%]	PD [mm]	rCAL/ CAL [mm]	CALG [mm]	rRH/ RGR [mm]	RGR reduction [mm]	KTH / KTW	KTT [mm]	RR	RC [%]	CLH [mm]	CLH [%]	CDC [%]
Santamaria et al.[24]	2013	fully restored	RMGIC	R + CTG	baseline	18.5	14.0	1.10 ±0.44	12.89 ±1.09	–	11.79 ±1.09	–	2.54 ±1.17	0.85 ±0.19	–	–	–	–	–
					6 months	–	–	2.15 ±0.67*	11.63 ±1.08*	–	9.48 ±0.82*	–	3.34 ±0.91*	1.95 ±0.42*	–	–	–	–	–
					1 year	–	–	2.12 ±0.56*	11.51 ±1.15*	–	9.51 ±0.88*	–	3.38 ±1.46*	1.81 ±0.50*	–	–	–	–	–
					2 years	–	–	2.11 ±0.78*	11.57 ±1.12*	–	9.57 ±0.81*	–	3.56 ±1.46*	1.87 ±0.72*	–	–	–	–	–
				CTG	baseline	19.4	18.0	1.15 ±0.48	12.85 ±2.06	–	11.70 ±2.01	–	2.38 ±1.22	0.90 ±0.23	–	–	–	–	–
					6 months	–	–	2.10 ±0.55*	11.27 ±1.17*	–	9.17 ±1.53*	–	3.05 ±1.11*	1.93 ±0.53*	–	–	–	–	–
					1 year	–	–	2.00 ±0.45*	11.15 ±1.72*	–	9.15 ±1.46*	–	3.17 ±1.50*	1.90 ±0.77*	–	–	–	–	–
					2 years	–	–	2.00 ±0.34*	11.27 ±1.70*	–	9.12 ±1.52*	–	3.20 ±1.00*	1.82 ±0.44*	–	–	–	–	–
Santamaria et al.[25]	2016	fully restored	NRC	RC + CTG	baseline	–	–	1.11 ±0.47	13.84 ±1.65	–	12.73 ±1.56	–	2.27 ±0.86	0.90 ±0.24	–	–	3.73 ±0.50	–	–
					6 months	–	–	2.77 ±0.42*	12.88 ±1.36*	–	10.10 ±1.29*	–	2.59 ±0.76*	2.00 ±0.30*	–	–	–	–	–
					1 year	–	–	2.66 ±0.48*	12.67 ±1.30*	1.17 ±0.89	10.01 ±1.30*	2.72 ±0.69	2.73 ±0.75*	1.97 ±0.26*	–	73.84 ±19.20	–	–	–
				CTG	baseline	–	–	1.16 ±0.38	13.41 ±2.00	–	12.24 ±1.90	–	2.41 ±1.20	0.90 ±0.23	–	82.16 ±16.10	3.39 ±0.57	–	–
					6 months	–	–	2.10 ±0.47	11.40 ±1.70*	–	9.31 ±1.60*	–	2.88 ±0.96*	1.87 ±0.47*	–	–	–	–	–
					1 year	–	–	2.00 ±0.48*	11.42 ±1.60*	1.98 ±0.81*	9.42 ±1.50*	2.82 ±0.74	3.00 ±0.90*	1.81 ±0.44*	–	82.16 ±16.10	–	–	–

Study	Year	Fully restored/ partially restored	Restoration used	Intervention	Follow-up	Outcome assessed														
						PI [%]	GI/ BOP [%]	PD [mm]	rCAL/ CAL [mm]	CALG [mm]	rRH/ RGR [mm]	RGR reduction [mm]	KTH / KTW	KTT [mm]	RR	RC [%]	CLH [mm]	CLH [%]	CDC [%]
Isler et al.[6]	2018	fully restored	NRC + RMGIC + giomer	NRC + CTG	baseline	–	–	1.13 ±0.34	12.5 ±0.88	–	11.37 ±0.73	–	3.17 ±1.15	0.89 ±0.12	–	–	3.07 ±1.13	–	71.31 ±21.73
					3 months	–	–	1.26 ±0.45	10.64 ±0.65	–	9.38 ±0.45	–	4.02 ±1.25	1.82 ±0.40	–	–	1.03 ±0.86	–	71.31 ±21.73
					6 months	–	–	1.30 ±0.47	10.79 ±0.66	–	9.40 ±0.45	–	3.76 ±1.02	1.70 ±0.38	–	–	1.03 ±0.81	–	69.86 ±20.82
					1 year	–	–	1.43 ±0.66	10.83 ±0.85	–	9.39 ±0.48	–	3.78 ±1.15	1.63 ±0.36	–	–	1.04 ±0.89	–	71.18 ±23.16
				RMGIC + CTG	baseline	–	–	1.13 ±0.46	12.48 ±0.89	–	11.35 ±0.73	–	3.30 ±0.99	0.89 ±0.12	–	–	2.89 ±1.20	–	
					3 months	–	–	1.13 ±0.34	10.63 ±0.63	–	9.50 ±0.43	–	3.87 ±0.98	1.82 ±0.41	–	–	1.04 ±1.08	–	68.85 ±21.19
					6 months	–	–	1.09 ±0.29	10.54 ±0.56	–	9.46 ±0.42	–	3.80 ±1.07	1.69 ±0.35	–	–	0.96 ±1.09	–	71.93 ±21.78
					1 year	–	–	1.17 ±0.39	10.59 ±0.62	–	9.41 ±0.39	–	3.83 ±1.10	1.68 ±0.33	–	–	1.00 ±1.04	–	71.33 ±22.33
				giomer + CTG	baseline	–	–	1.04 ±0.21	12.35 ±0.71	–	11.26 ±0.62	–	3.04 ±0.99	0.88 ±0.10	–	–	2.83 ±0.97	–	
					3 months	–	–	1.22 ±0.52	10.70 ±0.66	–	9.55 ±0.39	–	3.96 ±1.16	1.84 ±0.38	–	–	1.10 ±0.87	–	66.62 ±22.89
					6 months	–	–	1.22 ±0.42	10.79 ±0.74	–	9.53 ±0.37	–	3.72 ±1.16	1.71 ±0.33	–	–	1.10 ±0.86	–	65.79 ±22.09
					1 year	–	–	1.30 ±0.47	10.76 ±0.60	–	9.54 ±0.33	–	3.61 ±1.18	1.69 ±0.32	–	–	1.11 ±0.81	–	64.23 ±20.33
Santamaria et al.[8]	2018	partially restored vs. odontoplasty	RCom	PR + CTG	baseline	–	–	1.2 ±0.5	9.3 ±1.5	–	8.7 ±1.4	–	2.7 ±1.3	1.0 ±0.5	–	–	–	–	–
					6 months	–	–	2.5 ±0.5	8.8 ±1.0	–	6.3 ±1.7	–	4.1 ±0.9	2.1 ±0.6	–	–	–	–	–
					1 year	–	–	2.6 ±0.7	8.8 ±1.8	0.5 ±1.3	6.2 ±1.8	2.5 ±1.0	4.2 ±1.7	2.0 ±0.7	–	93.0 ±26.1	–	–	75.3 ±22.7
				CTG + odonto-plasty	baseline	–	–	1.3 ±0.5	10.5 ±1.5	–	9.2 ±2.5	–	2.9 ±0.9	0.9 ±0.2	–	–	–	–	–
					6 months	–	–	2.1 ±0.6	8.7 ±1.4	–	6.7 ±1.3	–	4.1 ±0.8	2.0 ±0.6	–	–	–	–	–
					1 year	–	–	2.0 ±0.5	8.8 ±2.0	1.7 ±1.4	6.8 ±1.9	2.4 ±1.1	4.1 ±1.1	1.9 ±0.6	–	92.2 ±28.4	–	–	74.6 ±31.5

Study	Year	Fully restored/ partially restored	Restoration used	Intervention	Follow-up	Outcome assessed													
						PI [%]	GI/ BOP [%]	PD [mm]	rCAL/ CAL [mm]	CALG [mm]	rRH/ RGR [mm]	RGR reduction [mm]	KTH / KTW	KTT [mm]	RR	RC [%]	CLH [mm]	CLH [%]	CDC [%]
Dursun et al.[26]	2018	fully restored	RMGIC + NIC	RMGIC + SCTG	baseline	0.17 ±0.25	0.23 ±0.23	1.73 ±0.64	3.16 ±0.65	–	3.50 ±1.04	–	2.83 ±1.85	1.22 ±0.54	–	–	–	–	–
					3 months	0.18 ±0.32*	0.06 ±0.14	1.63 ±0.40	1.47 ±0.76	–	0.44 ±0.70*	–	4.94 ±1.89*	–	–	–	–	–	–
					6 months	0.20 ±0.27	0.09 ±0.17	1.61 ±0.35	1.41 ±0.75	–	0.44 ±0.70	–	4.89 ±1.84*	–	–	–	–	–	–
					1 year	0.18 ±0.26*	0.06 ±0.17	1.88 ±0.29	1.76 ±0.76	1.66 ±0.76	0.44 ±0.70*	3.22 ±0.66	4.89 ±1.84*	2.30 ±0.08*	–	89.49 ±18.15	–	–	–
				NIC + SCTG	baseline	0.26 ±0.19	0.18 ±0.33	1.21 ±0.03	3.03 ±0.78	–	3.13 ±0.68	–	3.28 ±1.56	1.06 ±0.23	–	–	–	–	–
					3 months	0.42 ±0.33*	0.17 ±0.35	1.45 ±0.56	1.53 ±1.04	–	0.24 ±0.56*	–	5.92 ±1.44*	–	–	–	–	–	–
					6 months	0.40 ±0.28	0.14 ±0.28	1.37 ±0.43	1.53 ±0.96	–	0.24 ±0.56*	–	5.62 ±0.96*	–	–	–	–	–	–
					1 year	0.48 ±0.39*	0.12 ±0.27	1.28 ± 0.39	1.51 ± 0.86	1.61 ± 0.47	0.41 ± 0.71*	3.08 ± 0.71	5.62 ± 0.96*	2.16 ± 0.16*	–	90.12 ±16.58	–	–	–
				SCTG (control – RC without NCCL)	baseline	0.37 ±0.52	0.34 ±0.40	1.45 ±0.61	2.67 ±0.63	–	3.17 ±0.85	–	2.62 ±1.19	1.28 ±0.57	–	–	–	–	–
					3 months	0.35 ±0.34	0.26 ±0.33	1.50 ±0.55	1.16 ±0.64	–	0.06 ±0.23*	–	5.12 ±1.16*	–	–	–	–	–	–
					6 months	0.34 ±0.47	0.18 ±0.32	1.43 ±0.44	1.15 ±0.77	–	0.06 ±0.23*	–	5.12 ±1.16*	–	–	–	–	–	–
					1 year	0.25 ±0.31	0.12 ±0.22	1.48 ±0.41	1.25 ±0.57	1.35 ±0.76	0.06 ±0.23*	3.16 ±0.20	5.12 ±1.16*	2.36 ±0.18*	–	96.22 ±10.75	–	–	–
Ramireddy et al.[29]	2018	fully restored	RMGIC + PRF	RMGIC + CAF	baseline	–	–	1.21 ±0.41	11.74 ±1.25	–	10.54 ±1.29	–	2.23 ±0.43	2.13 ±0.11	–	–	–	–	–
					3 months	–	–	0.26 ±0.44	7.87 ±1.38	–	7.64 ±1.37	–	6.10 ±0.60	2.15 ±0.22	–	–	–	–	–
					6 months	–	–	0.21 ±0.41	7.77 ±1.40	–	7.59 ±1.39	–	6.18 ±0.68	2.19 ±0.12	–	–	–	–	–
				CAF + PRF	baseline	–	–	1.23 ±0.43	11.62 ±1.18	–	10.54 ±1.29	–	2.23 ±0.43	2.13 ±0.10	–	–	–	–	–
					3 months	–	–	0.23 ±0.43	7.92 ±1.21	–	7.69 ±1.15	–	6.00 ±0.76	2.66 ±0.14	–	–	–	–	–
					6 months	–	–	0.18 ±0.39	7.82 ±1.14	–	7.64 ±1.06	–	6.03 ±0.84	2.95 ±0.18	–	–	–	–	–

Study	Year	Fully restored/ partially restored	Restoration used	Intervention	Follow-up	Outcome assessed														
						PI [%]	GI/ BOP [%]	PD [mm]	rCAL/ CAL [mm]	CALG [mm]	rRH/ RGR [mm]	RGR reduction [mm]	KTH / KTW	KTT [mm]	RR	RC [%]	CLH [mm]	CLH [%]	CDC [%]
Lemos Reis et al.[30]	2020	fully restored	ADMG	CAF + ADMG (control)	baseline	–	–	1.5 ±0.6	4.4 ±1.1	–	3.1 ±0.2	–	2.8 ±0.6	1.0 ±0.5	–	–	–	–	–
					6 months	–	–	1.5 ±0.5	2.5 ±0.7	1.9 ±1.3	0.9 ±0.6	2.2 ±0.5	3.4 ±1.2	1.5 ±0.4	–	69.5 ±19.0	–	–	–
				CAF + ADMG (test)	baseline	–	–	1.5 ±0.6	4.8 ±1.3	–	3.3 ±0.4	–	2.6 ±0.8	1.0 ±0.4	–	–	–	–	–
					6 months	–	–	1.5 ±0.6	2.6 ±0.9	2.1 ±1.2	0.9 ±0.6	2.4 ±0.5	3.3 ±1.2	1.5 ±0.5	–	72.2 ±16.5	–	–	–
Cairo et al.[31]	2020	fully restored	composite	CAF	baseline	–	–	1.1 ±0.3	–	–	3.2 ±0.5	–	3.1 ±0.5	0.80 ±0.09	–	–	–	–	–
					6 months	–	–	1.1 ±0.3	–	–	0.3 ±0.5	–	3.3 ±0.5	–	2.9 ±0.7	69	–	–	–
					1 year	–	–	1.2 ±0.4	–	–	0.5 ±0.5	–	3.3 ±0.7	0.86 ±0.16	2.7 ±0.6	50	–	–	–
				CAF + CTG	baseline	–	–	1.1 ±0.3	–	–	3.4 ±0.6	–	2.9 ±1.1	0.78 ±0.12		–	–	–	–
					6 months	–	–	1.2 ±0.4	–	–	0.1 ±0.3	–	4.6 ±0.6	–	3.3 ±0.7	93	–	–	–
					1 year	–	–	1.1 ±0.3	–	–	0.3 ±0.5	–	4.6 ±0.5	1.38 ±0.09	3.1 ±0.7	71	–	–	–
Mathias-Santamaria et al.[27]	2022	partially restored	composite	CAF	baseline	–	–	1.5 ±0.5	11.8 ±1.7	–	3.2 ±0.5	–	2.1 ±1.0	1.0 ±0.5	–	–	–	–	–
					6 months	–	–	1.5 ±0.4	9.8 ±1.7	–	0.3 ±0.5	–	2.5 ±1.3	1.1 ±0.5	2.9 ±0.7	69	–	–	–
					1 year	–	–	1.5 ±0.4	9.8 ±1.6	–	0.5 ±0.5	–	2.5 ±1.2	1.1 ±0.4	2.7 ±0.6	50	–	–	–
				CAF + CM	baseline	–	–	1.5 ±0.4	12.6 ±1.6	–	3.4 ±0.6	–	2.4 ±1.5	1.0 ±0.6	–	–	–	–	–
					6 months	–	–	1.6 ±0.4	10.8 ±1.6	–	0.1 ±0.3	–	3.0 ±1.3	1.5 ±0.5	3.3 ±0.7	93	–	–	–
					1 year	–	–	1.6 ±0.4	10.8 ±1.5	–	0.3 ±0.5	–	3.3 ±1.2	1.7 ±0.6	3.1 ±0.7	71	–	–	–

Table 3 and Fig. 3 show the forest plot of the studies included in the meta-analysis. The point estimate is the effect size of PD between the interventions administered to the treatment and control arms. As can be observed from the figure, Isler et al.,[6] Santamaria et al.,[7,23,24] Dursun et al.,[26] Lucchesi et al.,[28] and Ramireddy et al.[29] reported a significant difference in the PD value between the 2 arms in their experiments.

Table 4 and Fig. 4 show the forest plot of the studies included in the meta-analysis. The point estimate is the effect size of KTH/KTT between the interventions administered to the treatment and control arms. As can be observed from the figure, Isler et al.,[6] Lucchesi et al.,[28] Ramireddy et al.,[29] and Lemos Reis et al.[30] reported a significant difference in the KTH/KTW value between the 2 arms in their experiments.

Table 5 and Fig. 5 show the forest plot of the studies included in the meta-analysis. The point estimate is the effect size of relative CAL(rCAL)/CAL between the interventions administered to the treatment and control arms. As shown in the figure, Isler et al.,[6] Santamaria et al.[7] and Ramireddy et al.[29] reported a significant difference in the rCAL/CAL value between the 2 arms in their experiments.

Table 6 and Fig. 6 show the forest plot of the studies included in the meta-analysis. The point estimate is the effect size of relative RH (rRH)/relative GR (RGR) between the interventions administered to the treatment and control arms. As seen in the figure, Isler et al.,[6] Santamaria et al.[7,23,24] and Ramireddy et al.[29] reported a significant difference in the rRH/RGR value between the 2 arms in their experiments.

Table 7 and Fig. 7 show the forest plot of the studies included in the meta-analysis. The point estimate is the effect size of the percentage RC between the interventions administered to the treatment and control arms.

Table 8 tabulates dentin sensitivity (DS) for the evaluation of patient-centered outcomes.

Table 9 and Fig. 8 show the effect size for the esthetic scores (ESs) obtained from the studies outlined in the meta-analysis. Changes in the visual analog scale (VAS) score for the esthetic outcome were observed for both treatment groups, and the differences between the effect sizes were used to compare the outcomes between the studies analyzed in the meta-analysis. As shown in Table 9, the summary score calculated showed a statistically significant difference in ES between the 2 groups assessed in each study.

Table 3. Forest plot of the effect size in the difference between the interventions in terms of pocket depth (PD) at 6 months

Study	PD (95% *CI*)	Weight
Isler et al.[6] (giomer + CTG)	0.47 (0.372–0.568)	10.2
Isler et al.[6] (RMGIC + CTG)	0.17 (0.134–0.205)	28.2
Santamaria et al.[7]	0.09 (0.067–0.112)	44.4
Santamaria et al.[8]	0.67 (0.564–0.776)	9.4
Santamaria et al.[22]	0.66 (0.508–0.811)	6.6
Santamaria et al.[23]	0.74 (0.623–0.857)	8.5
Santamaria et al.[24]	0.09 (0.075–0.105)	66.7
Santamaria et al.[25]	1.42 (1.183–1.667)	4.2
Dursun et al.[26] (RMGIC + SCTG)	0.41 (0.342–0.478)	14.6
Dursun et al.[26] (NIC + SCTG)	0.14 (0.117–0.163)	42.9
Mathias-Santamaria et al.[27]	0.10 (0.087–0.113)	78.7
Lucchesi et al.[28] (RMGIC + CPF)	0.40 (0.348–0.452)	19.2
Lucchesi et al.[28] (MRC + CPF)	0.20 (0.174–0.226)	38.4
Ramireddy et al.[29]	0.07 (0.059–0.093)	58.8
Cairo et al.[31]	0.33 (0.263–0.397)	14.8
Effects summary	0.42 (0.345–0.493)	–
Z-score (*p*-value)	0.254 (0.400)	–

CI – confidence interval.

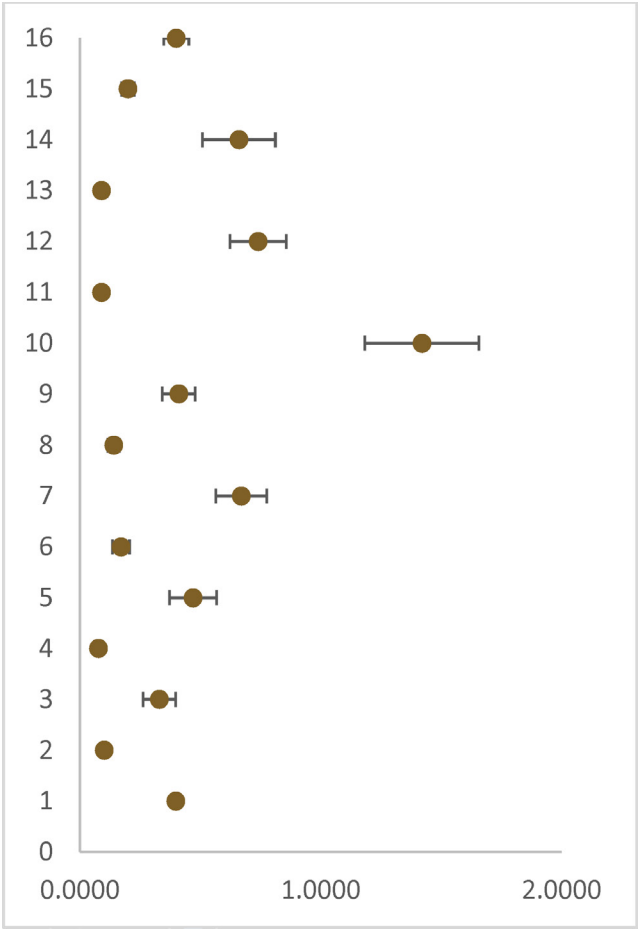

Fig. 3. Forest plot of the effect size in the difference between the interventions in terms of pocket depth (PD) at 6 months

Table 4. Forest plot of the effect size in the difference between the interventions in terms of keratinized tissue height (KTH)/keratinized tissue width (KTW) at 6 months

Study	KTH/KTW (95% *CI*)	Weight
Isler et al.[6] (RMGIC + CTG)	0.04 (0.032–0.048)	119.9
Isler et al.[6] (giomer + CTG)	0.04 (0.032–0.048)	119.9
Lucchesi et al.[28] (MRC + CPF)	0.14 (0.122–0.158)	54.9
Santamaria et al.[7]	0.26 (0.195–0.325)	15.4
Santamaria et al.[24]	0.26 (0.217–0.303)	23.1
Santamaria et al.[25]	0.30 (0.250–0.350)	20.0
Dursun et al.[26] (RMGIC + SCTG)	0.90 (0.750–1.050)	6.7
Dursun et al.[26] (NIC + SCTG)	0.43 (0.358–0.507)	14.0
Mathias-Santamaria et al.[27]	0.20 (0.174–0.225)	39.4
Ramireddy et al.[29]	0.18 (0.140–0.220)	24.8
Lemos Reis et al.[30]	0.08 (0.061–0.099)	51.5
Cairo et al.[31]	2.17 (1.727–2.613)	2.3
Effects summary	0.43 (0.347–0.507)	–
Z-score (*p*-value)	0.240 (0.405)	–

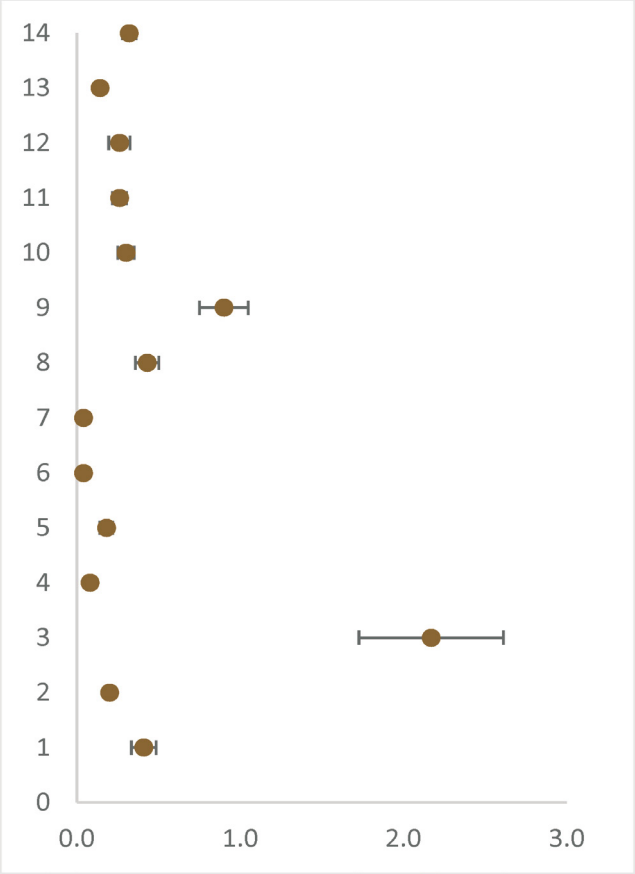

Fig. 4. Forest plot of the effect size in the difference between the interventions in terms of keratinized tissue height (KTH)/keratinized tissue width (KTW) at 6 months

Table 5. Forest plot of the effect size in the difference between the interventions in terms of relative clinical attachment loss (rCAL)/CAL at 6 months

Study	rCAL/CAL (95% *CI*)	Weight
Isler et al.[6] (RMGIC + CTG)	0.07 (0.059–0.081)	90.4
Isler et al.[6] (giomer + CTG)	0.38 (0.301–0.459)	12.6
Santamaria et al.[7]	0.08 (0.067–0.092)	79.1
Santamaria et al.[22]	0.21 (0.162–0.258)	20.8
Santamaria et al.[24]	0.21 (0.175–0.245)	28.6
Santamaria et al.[25]	0.87 (0.725–1.015)	6.9
Dursun et al.[26] (RMGIC + SCTG)	0.34 (0.283–0.397)	17.6
Dursun et al.[26] (NIC + SCTG)	0.49 (0.408–0.572)	12.2
Mathias-Santamaria et al.[27]	0.20 (0.175–0.225)	39.4
Lucchesi et al.[28] (MRC + CPF)	0.20 (0.174–0.226)	38.4
Ramireddy et al.[29]	0.04 (0.031–0.049)	111.8
Effects summary	0.28 (0.232–0.329)	–
Z-score (*p*-value)	0.261 (0.397)	–

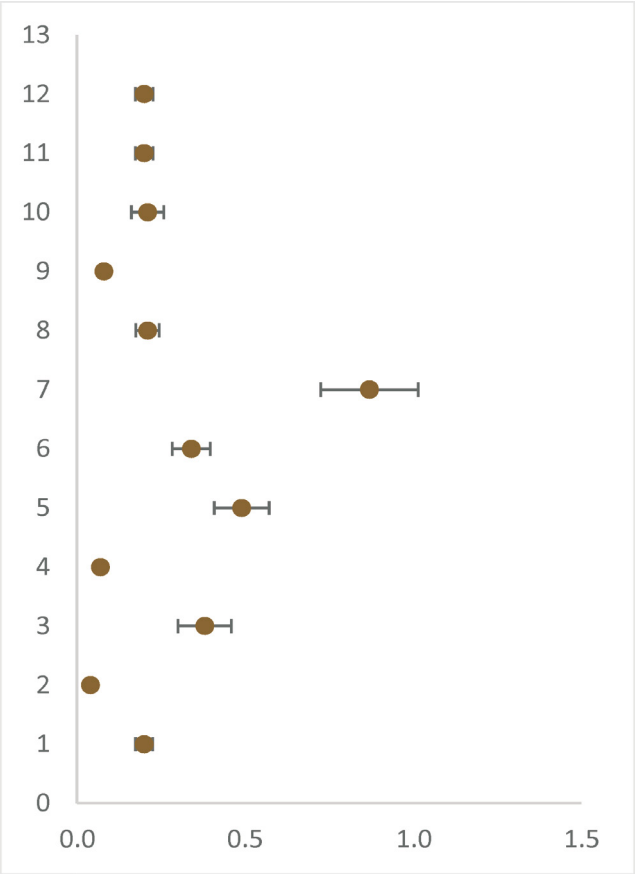

Fig. 5. Forest plot of the effect size in the difference between the interventions in terms of relative clinical attachment loss (rCAL)/CAL at 6 months

Table 6. Forest plot of the effect size in the difference between the interventions in terms of relative recession height (rRH)/relative gingival recession (RGR) at 6 months

Study	rRH/RGR (95% *CI*)	Weight
Isler et al.[6] (RMGIC + CTG)	0.13 (0.102–0.157)	36.9
Isler et al.[6] (giomer + CTG)	0.29 (0.229–0.35)	16.5
Santamaria et al.[7]	0.20 (0.15–0.25)	20.0
Santamaria et al.[8]	0.31 (0.26–0.359)	20.4
Santamaria et al.[23]	0.00 (0.003–0.004)	1,581.1
Santamaria et al.[24]	0.20 (0.166–0.233)	30.0
Santamaria et al.[25]	0.49 (0.408–0.571)	12.2
Dursun et al.[26] (RMGIC + SCTG)	1.65 (1.375–1.925)	3.6
Dursun et al.[26] (NIC + SCTG)	0.78 (0.65–0.91)	7.7
Mathias-Santamaria et al.[27]	0.40 (0.349–0.451)	19.7
Ramireddy et al.[29]	0.05 (0.038–0.061)	89.4
Effects summary	0.43 (0.353–0.510)	–
Z-score (*p*-value)	0.245 (0.403)	–

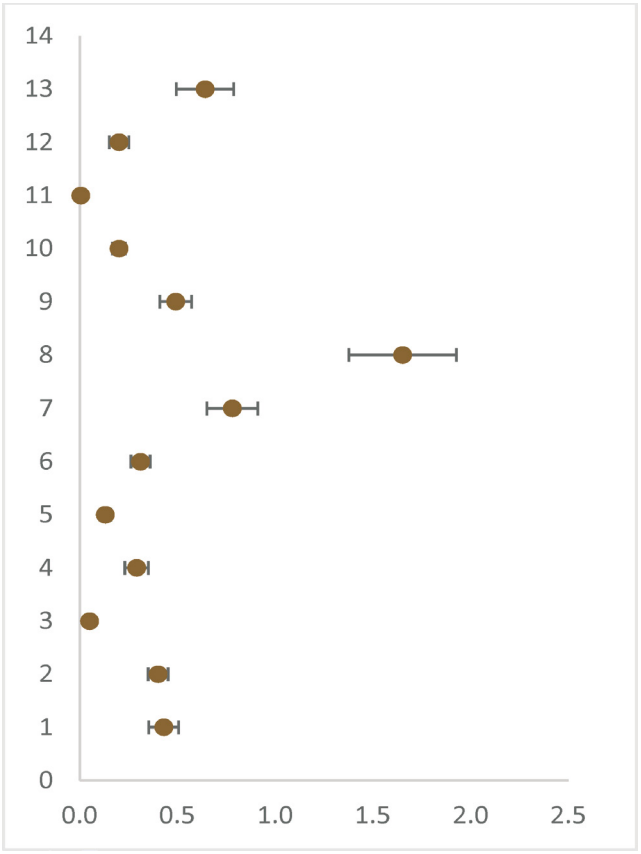

Fig. 6. Forest plot of the effect size in the difference between the interventions in terms of relative recession height (rRH)/relative gingival recession (RGR) at 6 months

Table 7. Forest plot of the effect size of the percentage root coverage (RC)

Study	%RC (95% *CI*)	Weight
Santamaria et al.[7]	0.064 (0.05–0.08)	62.1
Santamaria et al.[8]	0.029 (0.02–0.03)	215.6
Santamaria et al.[23]	0.133 (0.11–0.15)	47.6
Santamaria et al.[25]	0.470 (0.39–0.55)	12.8
Dursun et al.[26] (RMGIC + SCTG)	0.451 (0.38–0.53)	13.3
Dursun et al.[26] (NIC + SCTG)	0.437 (0.37–0.51)	14.5
Lemos Reis et al.[30]	0.152 (0.11–0.19)	27.2
Cairo et al.[31]	2.100 (1.67–2.53)	2.3
Effects summary	0.248 (0.20–0.29)	–
Z-score (*p*-value)	0.231 (0.408)	–

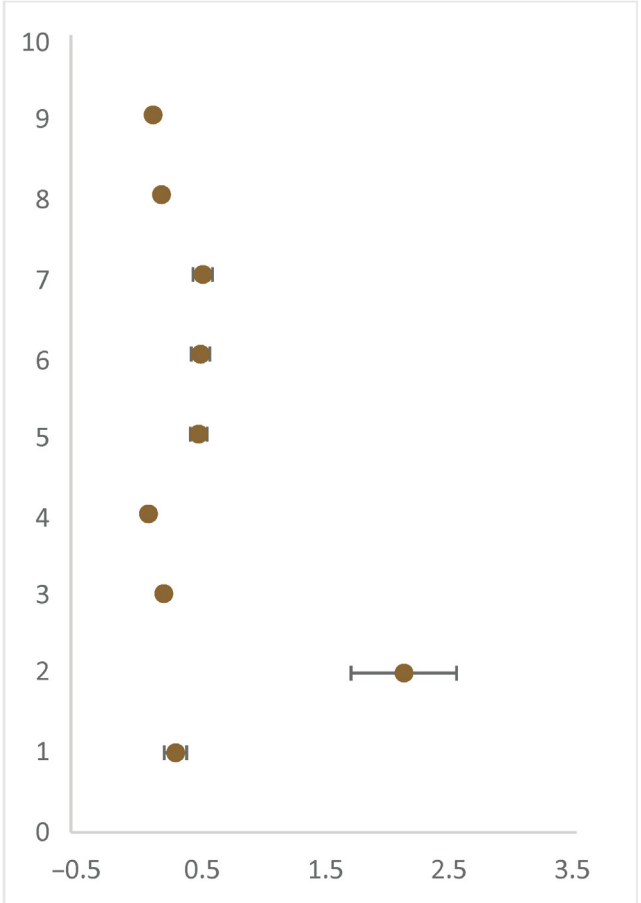

Fig. 7. Forest plot of the effect size of the percentage root coverage (RC)

Table 8. Dentin sensitivity (DS) in the included studies

Study	Year	Intervention	Follow-up	DS [%]	DS (VAS) *M* ±*SD*
Santamaria et al.[7]	2009	R + CAF	baseline	70	–
			6 months	5	–
		CAF	baseline	60	–
			6 months	35	–
Santamaria et al.[8]	2018	PR + CTG	baseline	–	3.7 ±3.3
			6 months	–	0.6 ±1.8
		CTG	baseline	–	5.0 ±3.1
			6 months	–	1.3 ±2.0
Santamaria et al.[22]	2008	R + CAF	baseline	68.42	–
			6 months	5.26	–
		CAF	baseline	68.42	–
			6 months	47.36	–
Santamaria et al.[25]	2016	RC + CTG	baseline	88	–
			6 months	6	–
		CTG	baseline	94	–
			6 months	44	–
Dursun et al.[26]	2018	RMGIC + SCTG	baseline	75.1 (n = 11)	–
			1 year	complain of sensitivity after 11 year (n = 1)	–
		NIC + SCTG	baseline	75.1 (n = 17)	–
			1 year	0	–
		SCTG (control – RC without NCCL)	baseline	75.1 (n = 13)	–
			1 year	0	–
Mathias-Santamaria et al.[27]	2022	CAF	baseline	–	4.9 ±3.6
			6 months	–	1.1 ±1.8
		CAF + CM	baseline	–	3.8 ±3.3
			6 months	–	1.1 ±2.3
Ramireddy et al.[29]	2018	RMGIC + CAF	6 months	83	–
		CAF + PRF	6 months	46	–
Cairo et al.[31]	2020	CAF	baseline	–	24.9 ±28.7
			6 months	–	1.4 ±5.5
			1 year	–	3.6 ±7.3
		CAF + CTG	baseline	–	29.1 ±29.6
			6 months	–	0.0 ±0.0
			1 year	–	1.9 ±4.9

Table 10 and Fig. 9 show the effect size for DS from the studies outlined in the meta-analysis. Changes in the VAS score for DS were observed for both treatment groups, and the differences between the effect sizes were used to compare the outcomes between the studies. Table 10 summarizes the score calculated from the studies and shows a statistically significant difference in DS between the 2 groups assessed in each study.

A summary of ESs is recorded in Table 11.

Table 12 and Fig. 10 show the risk ratio for DS obtained from the studies outlined in the meta-analysis. Change in the proportion of cases with DS were observed for both treatment groups, and the differences between the risk ratios were used to compare the outcomes between the studies. As seen in Table 12, the summary score calculated for the studies showed a statistically significant difference in DS between the 2 groups assessed in each study.

Table 9. Forest plot of the effect size in the difference between the interventions in terms of esthetic score (ES) after 1 year

Study	ES (95% *CI*)
Isler et al.[6] (RMGIC + CTG)	1.37 (1.08–1.66)
Isler et al.[6] (giomer + CTG)	1.32 (1.04–1.59)
Santamaria et al.[8]	0.10 (0.08–0.12)
Mathias-Santamaria et al.[27]	0.40 (0.35–0.45)
Effects summary	0.93 (0.74–1.12)
Z-score (*p*-value)	4.90 (0.500)

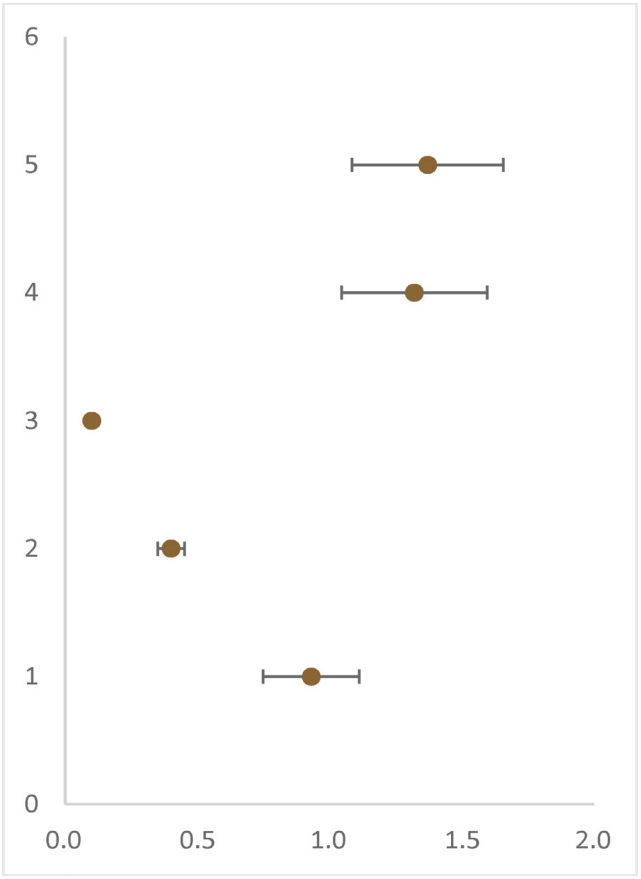

Fig. 8. Forest plot of the effect size in the difference between the interventions in terms of esthetic score (ES) after 1 year

Table 10. Forest plot of the effect size in the difference between the interventions in terms of dentin sensitivity (DS) – VAS (visual analog scale) score – after 1 year

Study	DS (VAS) (95% *CI*)
Isler et al.[6] (RMGIC + CTG)	0.56 (0.44–0.67)
Isler et al.[6] (giomer + CTG)	0.89 (0.71–1.08)
Santamaria et al.[8]	0.24 (0.20–0.28)
Mathias-Santamaria et al.[27]	1.10 (0.96–1.24)
Cairo et al.[31]	0.39 (0.31–0.47)
Effects summary	0.52 (0.41–0.62)
Z-score (*p*-value)	4.95 (0.500)

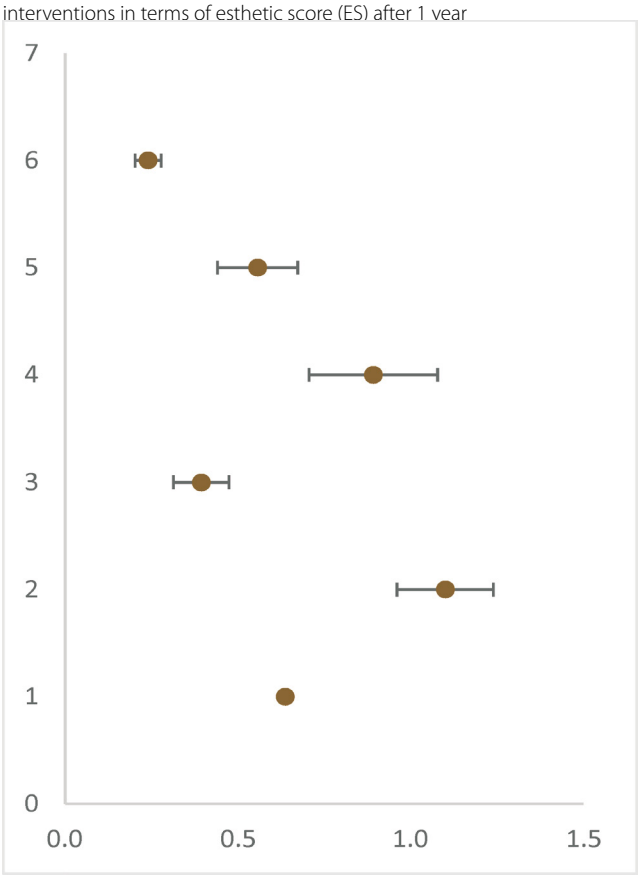

Fig. 9. Forest plot of the effect size in the difference between the interventions in terms of dentin sensitivity (DS) – VAS (visual analog scale) score – after 1 year

Table 11. Esthetic scores (ESs) in the included studies

Study	Year	Intervention	Follow-up	ES (VAS)	Satisfaction (VAS)
Isler et al.[6]	2018	NRC	baseline	3.02 ±1.24	–
			1 year	8.93 ±1.11	–
		RMGIC	baseline	3.65 ±1.33	–
			1 year	8.52 ±1.33	–
		giomer	baseline	3.36 ±1.28	–
			1 year	8.57 ±1.53	–
Santamaria et al.[8]	2018	CTG	baseline	4.1 ±2.9	–
			6 months	9.0 ±2.3	–
			1 year	9.2 ±1.1	–
		PR + CTG	baseline	4.6 ±2.3	–
			6 months	9.1 ±2.2	–
			1 year	9.1 ±1.0	–
Mathias-Santamaria et al.[27]	2022	CAF	baseline	3.4 ±2.6	–
			6 months	8.9 ±1.1	–
			1 year	9.0 ±0.9	–
		CAF + CM	baseline	3.9 ±2.7	–
			6 months	9.0 ±1.2	–
			1 year	9.2 ±1.1	–
Cairo et al.[31]	2020	CAF	baseline	–	–
			6 months	–	–
			1 year	91.2 ±9.8	95.4 ±6.0
		CAF + CTG	baseline	–	–
			6 months	–	–
			1 year	88.6 ±10.4	90.9 ±10.7

Table 12. Forest plot of the effect size in the difference between the interventions in terms of dentin sensitivity (DS) – risk ratios – after 1 year

Study	Risk ratio for DS (95% *CI*)
Santamaria et al.[7]	12.29 (9.22–15.36)
Santamaria et al.[22]	11.57 (8.92–14.22)
Santamaria et al.[25]	12.55 (10.46–14.64)
Effects summary	12.14 (9.53–14.74)
Z-score (*p*-value)	4.26 (<0.001)

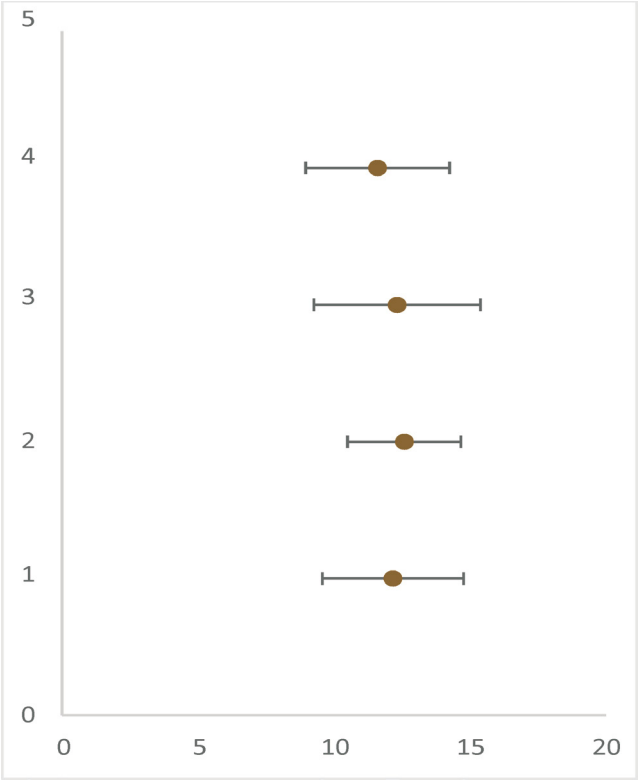

Fig. 10. Forest plot of the effect size in the difference between the interventions in terms of dentin sensitivity (DS) – risk ratios – after 1 year

Results

A total of 222 potentially relevant papers were identified through the search strategy, of which 199 were excluded after screening the titles and abstracts. The full texts of 23 papers were assessed based on the inclusion and exclusion criteria, with 13 articles fulfilling the eligibility criteria and included in the review. The reasons for the exclusion of 10 articles are shown in Fig. 1.

Included studies

A total of 428 patients were enrolled in the 13 included articles, 12 studies[6,7,8,22,23,25–31] completed the follow-up periods and 1 RCT[24] reported dropouts. The characteristics of the studies are shown in Table 1.

Age groups

The age of patients ranged from 19 to 71 years. Four RCTs[7,22–24] included participants with Miller's class 1 GR, 3 RCTs[8,25,29] included Miller's class 1 and 2 GR, and 5 studies[6,26,28,30,31] did not mention the GR classification. One study by Mathias-Santamaria et al.[27] included GR based on a different classification system[32] (a single recession type (RT)-1[32] associated with class B+ or B– NCCL,[33] forming a combined defect on a vital canine or premolar).

Follow-up

The maximum follow-up period for 5 RCTs[7,22,28–30] was 6 months, 6 RCTs[6,8,25–27,31] followed up for 1 year, and Santamaria et al.[23] and Santamaria et al.[24] for 2 years.

Study design

Three studies[22,23,30] had a split-mouth design, while the others used a parallel group design. Eleven RCTs[6,7,22–26,28–31] performed complete NCCL restoration, whereas 2 – by Santamaria et al.[8] and Mathias-Santamaria et al.[27] employed partial restoration.

Type of material used

Various materials were used for NCCL restoration, with 4 studies[6,26,28,29] using RMGIC, and others employing giomer,[6] nano-ionomer cement (NIC),[26] micro-filled resin composite (MRC),[28] or nano-filled resin composite (NRC) alone[25,27] or in combination.[6,26] However, no evidence suggested that the material type affected the surgical outcome.

Type of surgical root coverage procedure

Seven studies[7,22,27–31] performed the coronally advanced/positioned flap and 6 studies[6,8,23–26] used CTG for RC.

Esthetic scores

Five studies[6,8,26,27,31] recorded ES and reported that the restoration of NCCLs in combination with any RC procedure provided better esthetic results. Dursun et al.[26] also reported that ES was similar in the RMGIC and NIC groups (9.06 ±1.43). Two studies involved professional esthetic assessment by recording the root coverage esthetic score (RES)[31] and the modified root coverage esthetic score (mRES).[27]

Dentin hypersensitivity

Three of the RCTs[6,27,31] recorded the VAS scores for DS and reported decreased dentin hypersensitivity as perceived by the patient. Cairo et al.[31] recorded the VAS scores for patient satisfaction at 1 year and reported no significant difference between the 2 groups.

Six studies[7,22,25,26,29,31] reported the percentage of sites with DS. All of these studies reported a statistically significant difference in the percentage of dentin hypersensitivity, and there was a more significant decrease in the percentage of dentin hypersensitivity in the restored NCCL group.

Discussion

From among the 13 included studies, only 3 RCTs[6,7,29] compared the restored NCCLs with the unrestored NCCLs. All 13 studies demonstrated that the restoration of NCCLs had a significant impact on PD, rCAL/CAL and rRH/RGR.

Complete root coverage (CRC) was the most used and indicated primary outcome, as it is the main objective of RC procedures. Nonetheless, there was no significant difference in RC when comparing GR without NCCL with the GR of the root surface with NCCL, suggesting that root surface restoration did not markedly affect the outcome of the surgical procedure.

In RC procedures using soft tissue augmentation, such as platelet-rich fibrin (PRF),[29] CTG[6,8,23–26] or the acellular dermal matrix,[30] there was an increase in KTT. However, there was no significant difference in the outcome of the surgical procedure in terms of RC.

No data indicated the sequence of restoration, or whether restoration should be complete or partial, with only one study analyzing partial restoration.[27] Most of the studies employed restoration before surgical procedures, maybe due to better isolation conditions.

The risk of bias is an indicator of the methodological quality of the studies included. As shown in Fig. 2, the studies had a low risk of bias. In these studies, it was not possible to attain blinding or masking, as it was easy to notice if the NCCL was restored.

Conclusions

In the teeth with NCCLs and GR, the restoration of NCCL does not affect the percentage RC. However, it significantly decreases dentin hypersensitivity, and the patient's perception of esthetics and satisfaction.

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

Application of neural networks for the detection of oral cancer: A systematic review

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Abstract

One potential application of neural networks (NNs) is the early-stage detection of oral cancer. This systematic review aimed to determine the level of evidence on the sensitivity and specificity of NNs for the detection of oral cancer, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and Cochrane guidelines. Literature sources included PubMed, ClinicalTrials, Scopus, Google Scholar, and Web of Science. In addition, the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool was used to assess the risk of bias and the quality of the studies. Only 9 studies fully met the eligibility criteria. In most studies, NNs showed accuracy greater than 85%, though 100% of the studies presented a high risk of bias, and 33% showed high applicability concerns. Nonetheless, the included studies demonstrated that NNs were useful in the detection of oral cancer. However, studies of higher quality, with an adequate methodology, a low risk of bias and no applicability concerns are required so that more robust conclusions could be reached.

Keywords: oral cancer, oral neoplasms, medical informatics applications, computer neural networks, cancer early detection

Introduction

Artificial intelligence (AI) has significantly impacted the field of medicine,[1] and much AI research focuses on the diagnosis and prognosis of cancer,[2] neurological disorders[3] and cardiovascular diseases,[4] among others.[5] Neural networks (NNs) constitute an area of AI. They contain sets of artificial neurons organized in superimposed layers – an input layer, *n* intermediate layers for data processing and a result layer.[6] Deep learning (DL) is a combination of NN and machine learning; it enables the creation of computational models composed of multiple processing layers, able to learn the representations of data with multi-level abstraction.[7] In DL, convolutional, recursive and recurrent NNs have been applied.[8] Convolutional neural networks (CNNs) are a class of DL algorithms applied to medical image classification,[9] including those used for cancer detection.[10–13]

Oral cancer ranks sixth among the most common high-risk malignancies in middle-income countries globally.[14] The most common type of oral cancer is oral squamous cell carcinoma (OSCC).[15] Early diagnosis and treatment are crucial to improve patient survival. The histopathological examination of biopsy samples is the gold standard in diagnosing oral cancer. However, this approach is invasive and the samples require complex processing.[16] The detection of oral cancer in situ results in survival rates as high as 82%, though these rates can decrease to 32% if metastases are detected.[17] Therefore, an early diagnosis is essential, and recommendations state that any suspicious lesion that does not heal within 15 days after detection and the removal of the local causes of irritation should be biopsied.[18,19] Although the histopathological examination of biopsy specimens is the current reference method,[20] there are still discrepancies (12%) between the initial diagnosis from the incisional biopsy and the final histopathology results following the excision of the lesion.[21] However, many patients are reluctant to have a suspicious lesion biopsied by a clinician, for various reasons, including cost, fear of the procedure, concerns about healing, and esthetics. As a result, patients often postpone the biopsy to get a second opinion on its necessity. Therefore, research groups have proposed other diagnostic methods that are logistically more accessible. One of such approaches is the use of NNs for the early diagnosis of oral cancer through the analysis of risk factors, laboratory tests and the images of the lesion.[22,23]

The process of detection of oral cancer through imaging has different phases. Ideally, during the training phase, a set of images classified into different types, such as the normal region, the cancerous region and the precancerous region, is introduced to NN. The classified images allow NN to learn the characteristics of each set of images. Subsequently, in the testing phase, the image of a suspicious oral lesion is provided and the system outputs the predicted result.[24] Therefore, the NN diagnosis of oral cancer can be made by clinicians working in remote areas, where biopsy processing is complicated.

For the reasons outlined above, this systematic review aimed to determine the level of evidence on the sensitivity and specificity of NNs for detecting oral cancer.

Material and methods

Study protocol registration

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Diagnostic Test Accuracy Studies (PRISMA-DTA) statement[25] and the Cochrane guidelines.[26] The protocol was registered in the International Prospective Registry of Systematic Reviews (PROSPERO) (CRD42021256938). The articles included in the present systematic review were studies with an observational design (cohort and case–control studies). Case reports, case series, animal studies, pilot studies, short communications, and systematic reviews were excluded.

Eligibility criteria, information sources and search strategy

The eligibility of the studies was determined using the modified PICO strategy (Patient/Population, Intervention, Comparison, and Outcome). Searches with no restriction on the publication date were carried out in PubMed, ClinicalTrials, Scopus, Google Scholar, and Web of Science in April 2021, and were updated in July 2022. The search strategies used for each database are shown in Table 1. A manual search was performed by reading the reference sections of the included studies.

To meet the eligibility criteria, studies needed to use NN for the analysis of images for the detection of oral cancer in humans, and assess the sensitivity, specificity, precision, and accuracy of NN in comparison with the histopathological examination. All studies that used NN for the prognosis of oral cancer, to determine the efficacy of oral cancer treatment or to classify the stages of oral cancer, as well as studies that used other methods of data collection (not imaging), were excluded.

Study selection

For the selection of studies, the title and abstract of each paper were read. Those which answered the research question were reviewed in full text to determine if they met the eligibility criteria. If the eligibility criteria were not met, the articles were eliminated with reasons, as shown in Fig. 1.

Table 1. Keywords and algorithms used in the search strategy for each database

PICO strategy	Keywords
Population	patients with suspicious lesions or oral cancer
Intervention (diagnosis)	NN
Comparison	histopathological examination or clinical assessment
Outcome	sensitivity, specificity, precision, and accuracy, correlation coefficient, ROC curve, AUC
Study design	observational
Restrictions	in English or Spanish
Electronic database	PubMed, Clinical Trials, Scopus, Google Scholar, Web of Science
Focus question	What is the evidence on the use of NN for oral cancer detection?
Databases and registries	**Algorithm**
Google Scholar	("detection") + ("mouth neoplasm" OR "neoplasm, mouth" OR "malignant oral lesions" OR "tongue squamous cell carcinoma" OR "oral cancer" OR "cancer, oral" OR "submucous fibrosis" OR "oral submucous fibrosis") + ("deep learning" OR "neural network")
PubMed	("mouth neoplasm" OR "neoplasm, mouth" OR "malignant oral lesions" OR "tongue squamous cell carcinoma" OR "oral cancer" OR "cancer, oral" OR "submucous fibrosis" OR "oral submucous fibrosis") AND ("hierarchical learning" OR "deep learning" OR "neural network" OR "computer neural network" OR "computer neural networks" OR "network, computer neural" OR "networks, computer neural" OR "neural network, computer")
Clinical Trials	oral cancer + neural network
Scopus	("mouth neoplasm" OR "neoplasm, mouth" OR "malignant oral lesions" OR "tongue squamous cell carcinoma" OR "oral cancer" OR "cancer, oral" OR "submucous fibrosis" OR "oral submucous fibrosis") AND ("hierarchical learning" OR "deep learning" OR "neural network" OR "computer neural network" OR "computer neural networks" OR "network, computer neural" OR "networks, computer neural" OR "neural network, computer")
Web of Science	("mouth neoplasm" OR "neoplasm, mouth" OR "malignant oral lesions" OR "tongue squamous cell carcinoma" OR "oral cancer" OR "cancer, oral" OR "submucous fibrosis" OR "oral submucous fibrosis") AND ("hierarchical learning" OR "deep learning" OR "neural network" OR "computer neural network" OR "computer neural networks" OR "network, computer neural" OR "networks, computer neural" OR "neural network, computer")

NN – neural network; ROC curve – receiver operating characteristic curve; AUC – area under the ROC curve.

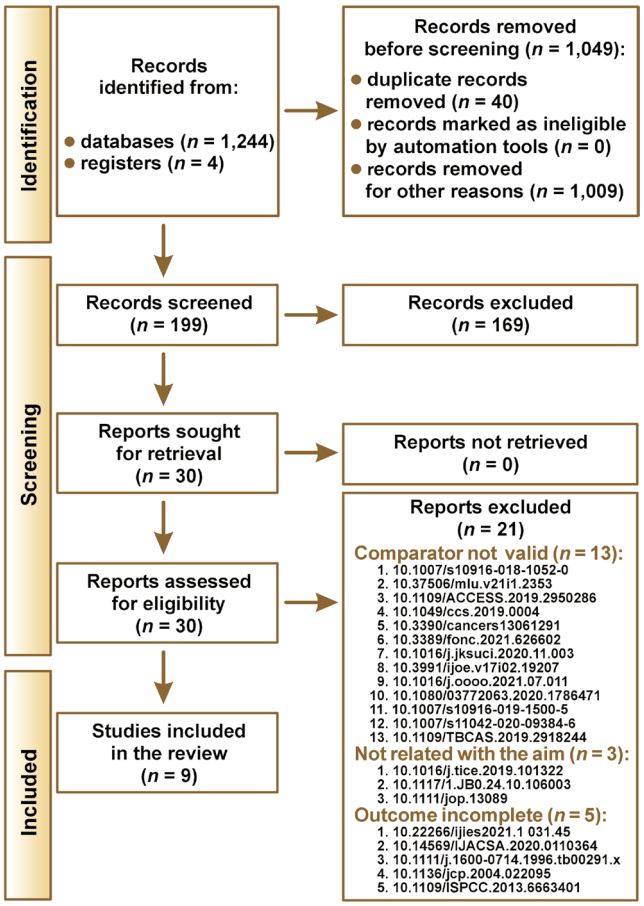

Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow chart

Data collection and data extraction

The relevant data from the selected articles was extracted, processed and tabulated using a Microsoft Excel spreadsheet. Data extraction was performed independently by 2 reviewers (M.P.B.-C. and M.E.M.C.-G.).

Data synthesis

The results were formally synthesized by grouping the data according to the type of images used for cancer detection, which included photographic images, confocal laser endomicroscopy (CLE), hyperspectral imaging (HSI), optical coherence tomography (OCT), and high-resolution microendoscopy (HRME). The summary of the individual studies with the details of the relevant data, such as the type of images, the NN computing technique, comparators, and outcomes, are presented in the result tables.

Synthesis of the results

If the results of the studies showed high heterogeneity in methodological or population characteristics, a synthesis without a meta-analysis (SWiM) was performed using the qualitative synthesis[27] and a representative graph.

Risk of bias and applicability

Two reviewers (R.T.-R. and L.A.-F.) assessed the risk of bias and the applicability of each study, using

the modified Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool,[28] which includes the patient selection, index test, reference standard, and flow and timing domains (Table 2). Any disagreement in the assessment of the risk of bias was resolved by the consensus of the research group.

Results

Study selection and study characteristics

The search of electronic databases and registries generated 1,248 records, of which 40 duplicate records were eliminated. After reading the titles and abstracts, it turned out that 30 articles answered the research question, and thus their full texts were retrieved. Subsequently, it was determined if they met the eligibility criteria, which resulted in the reasonable exclusion of 21 articles. For the qualitative analysis, 9 articles were included (Fig. 1). The characteristics of each of the studies and the extracted data are shown in Table 3. The synthesis of the results without a meta-analysis is shown in Fig. 2.

Synthesis of the results

Studies detecting oral cancer used various image types, including photographic images, CLE, HSI, OCT, and HRME.

Photographic images were used most frequently for the detection of oral cancer. Welikala et al. acquired images with a cell phone camera at the primary clinical care level[22]; Jubair et al. used various types of digital cameras and smartphones.[29] Welikala et al., who used ResNet-101 for image classification and Faster R-CNN for object detection, reported a precision of 84.77% and a recall

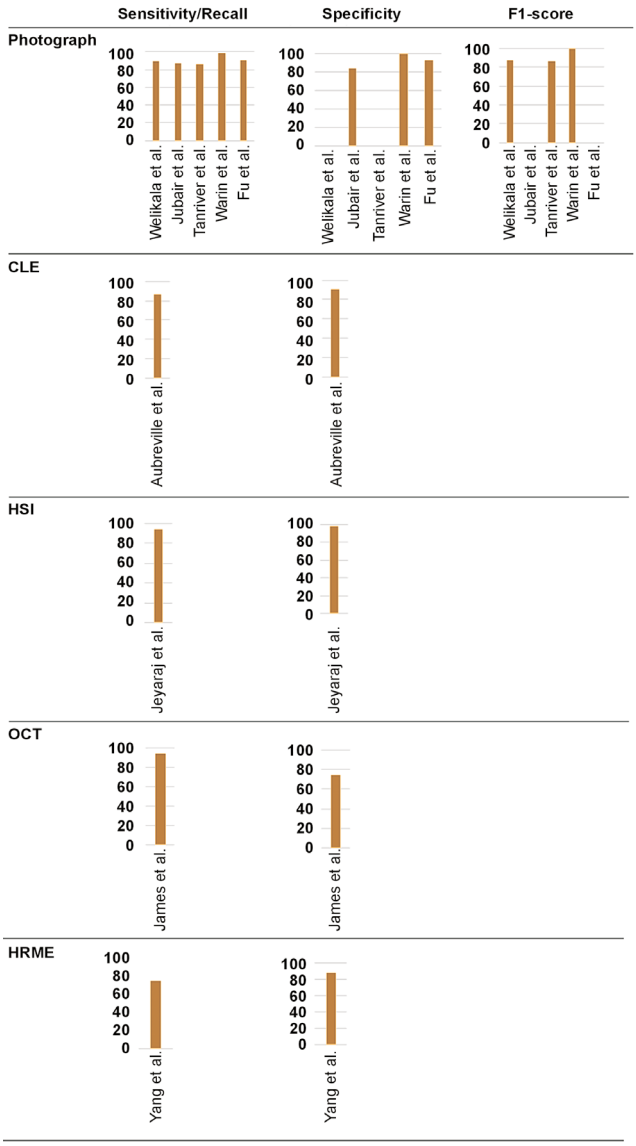

Fig. 2. Graphical representation of the synthesis of the results without a meta-analysis

Table 2. QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies 2) modified for the review

Domain		Questions
Risk of bias	1. Patient selection	Was a consecutive or random sample of patients enrolled?
		Was a case–control design avoided?
		Did the study avoid inappropriate exclusions?
	2. Index test	Do the datasets for training and testing contain at least 100 images for each classification to be evaluated?*
		Is the ratio of images used for training to patients no greater than 3:1*
		Is the testing dataset separate from the training dataset?*
	3. Reference standard	Is the reference standard likely to classify the target condition correctly?
		Were the reference standard results interpreted without the knowledge of the results of the index test?
	4. Flow and timing	Was there an appropriate interval between the index test and the reference standard?
		Did all patients receive the same reference standard?
		Were all patients included in the analysis?
Applicability	1.Patient selection	Are there concerns that the included patients and the setting do not match the review question?
	2. Index test	Are there concerns that the index test, its conduct or interpretation do not match the review question?
	3. Reference standard	Are there concerns that the target condition as defined by the reference standard does not match the review question?

* element modified according to the review topic.

Table 3. Characteristics of the included studies and their results

Type of images	Study	Data type or population	NN	Results
Photographic images	Welikala et al. 2020[22]	oral cavity images from cell phone camera testing: 204 images	ResNet-101 Faster R-CNN	multi-class image classification results lesion: precision – 84.77% recall – 89.51% F1 score – 87.07%
	Jubair et al. 2022[29]	photographic images of various tongue lesions testing: 100 images	EfficientNet-B0	AUC = 0.928 sensitivity – 86.7% specificity – 84.5% accuracy – 85.0%
	Tanriver et al. 2021[30]	photographic images of oral lesions testing: 69 images	CNN architecture: YOLOv5 EfficientNet-B4	benign class: precision – 89% recall – 86% F1-score – 88% support – 29 OPMD class: precision – 74% recall – 87% F1-score – 90% support – 23 carcinoma class: precision – 100% recall – 82% F1-score – 90% support – 17 weighted average: precision – 87% recall – 86% F1-score – 86% support – 69
	Warin et al. 2021[31]	clinical oral photographs collected retrospectively testing: 140 images	CNN with DenseNet-121 and Faster R-CNN	classification with DenseNet-121: precision – 99.00% sensitivity – 98.75% specificity – 100% F1-score – 99.00% detection accuracy with Faster R-CNN: precision – 76.67% recall – 82.14% F1-score – 79.31%
	Fu et al. 2020[32]	photographs of biopsy-proven OSCC and normal controls clinical validation: 666 images external validation: 402 images	automated DL algorithm using cascaded CNNs	clinical validation: AUC = 0.970 sensitivity – 91.0% specificity – 93.5% accuracy – 92.3%
CLE	Aubreville et al. 2017[34]	116 video sequences of a suspicious carcinogenic region 12 patients	CNN-based approaches	AUC = 0.960 sensitivity – 86.6% specificity – 90.0% accuracy – 88.3%
HSI	Jeyaraj et al. 2019[36]	HSI images of oral cancer training: 500 images	CNN with 2 partitioned layers for labeling and classifying the region of interest in multidimensional HSI	for 500 training patterns: sensitivity – 94% specificity – 98% accuracy – 94.5%
OCT	James et al. 2021[23]	OCT images validation: 1,078 images	14 pre-trained NN best results with DenseNet-201 and NASNetMobile	delineating cancer: sensitivity – 93% specificity – 74%
HRME	Yang et al. 2020[41]	HRME images testing: 253 images	U-Net	sensitivity – 75% specificity – 89% accuracy – 86%

CLE – confocal laser endomicroscopy; HSI – hyperspectral imaging; OCT – optical coherence tomography; HRME – high-resolution microendoscopy; OSCC – oral squamous cell carcinoma; CNN – convolutional neural network; DL – deep learning; OPMD – oral potentially malignant disorder.
F1 score = 2 × (precision × recall) / (precision + recall)

of 89.51%.[22] Jubair et al. used a pre-trained EfficientNet-B0 as a lightweight transfer learning model for oral cancer detection, and reported a sensitivity of 86.7% and a specificity of 84.5%.[29] On the other hand, Tanriver et al. collected photographic images of oral lesions with histopathological results from the archive of the Department of Tumor Pathology of the Oncology Institute at Istanbul University, Turkey; the rest of the images were collected from publicly available sources by using search engines (https://images.google.com and https://yandex.com/images).[30] The dataset comprised a diverse set of lesions coming from a wide range of oral diseases and anatomical regions. The authors reported a precision of 87% and a recall of 86%.[30] Likewise, Warin et al. retrospectively collected clinical oral photographs obtained between 2009 and 2018 at an oral and maxillofacial surgery center.[31] They used DenseNet-121 for classification, and reported a sensitivity of 98.75% and a specificity of 100%.[31] Finally, Fu et al. used biopsy-confirmed OSCC photographs from 11 hospitals in China, and reported a sensitivity of 91.0% and specificity of 93.5%.[32]

Confocal laser endomicroscopy is an adaptation of the conventional optical microscopy technique, in which the light from a laser source directed at a pinhole geometrically removes information from the outside of the focal plane and generates an optical plane at a specific depth from the surface.[33] Aubreville et al. used 16-bit grayscale CLE images to analyze 4 regions of interest, including the inner lower lip, the upper alveolar ridge and the hard palate.[34] The images acquired from suspicious lesions and 3 other areas that were assumed to be healthy resulted in a sensitivity of 86.6% and a specificity of 90.0%.[34]

Hyperspectral imaging acquires a three-dimensional (3D) data set called a hypercube, formed by 2 spatial dimensions and 1 spectral dimension. Using HSI provides information on tissue physiology, morphology and composition. One field of application for HSI is image classification for detecting tissues at risk of cancer.[35] Jeyaraj et al. applied a novel CNN with 2 partitioned layers to label and classify the region of interest in multidimensional HSI, and reported a sensitivity of 94% and a specificity of 98%.[36]

Optical coherence tomography is a non-invasive high-resolution optical imaging technology that produces real-time cross-sectional images in two-dimensional (2D) space (a lateral coordinate and an axial coordinate).[37] It is analogous to ultrasound imaging, except it uses light instead of sound, and is a powerful imaging technology for medical diagnosis, acting as a type of optical biopsy. However, unlike the conventional histopathological examination, which requires the extraction and processing of a tissue sample for microscopic evaluation, OCT can generate real-time images of the tissue.[38] James et al. used OCT images to classify non-dysplasia, dysplasia and malignancy through artificial NN/machine learning, and reported a sensitivity of 93% and a specificity of 74% for OSCC identification.[23]

High-resolution microendoscopy enables real-time epithelial imaging with subcellular resolution. Numerous research studies on gastrointestinal neoplasms has indicated that HRME is a modality that provides high specificity and precision for diagnosing different diseases.[39,40] Yang et al. developed an algorithm to determine whether HRME images show enough oral epithelial nuclei to differentiate between oral cancer and benign tissue.[41] Their study used 811 HRME images from 169 patients and demonstrated that HRME images were suitable for classifying oral cancer. The researchers reported a sensitivity of 75% and a specificity of 89%.[41]

Assessment of the risk of bias and applicability

Regarding domain 1 (patient selection), all studies exhibited a high risk of bias, with the main issues being an inadequate selection of patients and a lack of investigator blinding. Furthermore, 1 study (11%) had a high risk of bias with regard to domain 2 (index test), as it used a small number of images.[34] As many as 33% of the articles showed high applicability concerns (Fig. 3).

Discussion

In this systematic review, the type of NN applied for the detection of oral cancer was analyzed. All studies used CNN, probably due to the ease of working with images. The comprehensive search aimed to identify whether the studies used an additional type of NN to support the oral cancer detection process. In this regard, Sharma and Om developed a probabilistic NN and general regression model for the early detection and prevention of oral cancer, using various indicators, such as clinical symptoms, medical history and personal history.[42] This review identified an area of opportunity, which involves using CNN and other types of NN in the analysis of risk factors to provide a more reliable diagnosis of oral cancer, as this combination of data has not been assessed so far.

When verifying the accuracy of the algorithms used for oral cancer diagnosis, the images used for training must come from patients with the diagnosis confirmed through the histopathological examination. Studies were excluded if they did not report the gold standard for the validation of diagnosis, since the absence of an adequate comparator invalidates the results of such studies.

In the study by Tanriver et al., the training, validation and testing dataset was inadequate.[30] They obtained some of the images from a hospital (validated by a histopathologist), but as the sample was insufficient, they sourced other images through searching publicly accessible repositories.[30] However, such images do not provide the certainty of histopathological diagnostic validation.

Several studies tested the effect of the sample size during the training phase. Narayana et al. determined that a sample size of at least 50 was necessary.[43] Fang et al. conducted

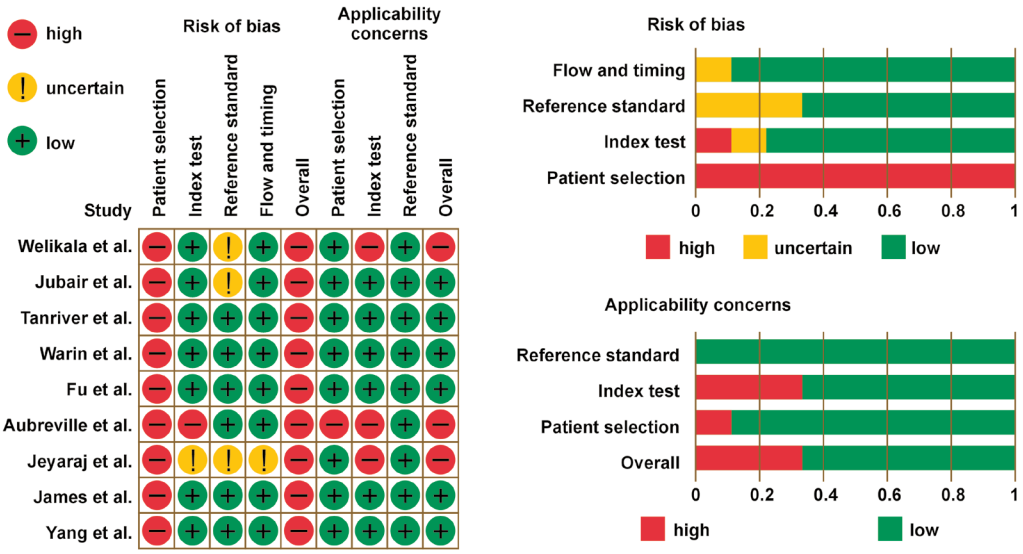

Fig. 3. Graphical representation of the risk of bias and the applicability of the included studies, obtained by means of the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies 2) evaluation

a study that aimed to investigate the impact of the training sample size on the performance of organ self-segmentation (Eye L, Eye R, Lens L, Lens R, Optic nerve L, Optic nerve R, Parotid L, Parotid R, Spinal cord, Larynx, and Body) in computed tomography (CT) based on DL for head and neck cancer patients.[44] They found that 200 samples were required to obtain a 90% yield for lenses and optic nerves, whereas the remaining organs needed at least 40 images for their detection.[44] However, according to Narayana et al., the minimum training sample size depends on a number of factors, such as the acquisition protocol, the type of tissue to be segmented, and others.[43] The results are not only associated with the dataset, but also with the specific CNN configuration.[43] According to Samala et al., assessing the precision and accuracy of CNN architecture by using a test set may be overly optimistic.[45] Therefore, validating the training process with unknown and independent cases derived from actual clinical practice is crucial. So far, no studies have tested the algorithms developed in this way.

Artificial intelligence can support the detection of cancer in its early stages. The evidence on the efficacy of CNN in image-based oral cancer detection demonstrated that NN could be used in daily clinical practice using photographs. This could be particularly helpful for clinicians in remote locations, where access to specialist oral pathology advice is limited.

Conclusions

Convolutional neural networks can potentially detect oral cancer in its early stages, though the results need to be verified by the corresponding histopathological examination. Most of the analyzed studies showed an accuracy greater than 85%. However, several studies encountered training problems due to the reduced number of images or because the testing process was performed on the same samples and not in clinical practice. In addition, the analysis of patient-specific risk factors and habits should complement these applications to formulate a more accurate diagnosis.

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

Can smoking alter salivary homeostasis? A systematic review on the effects of traditional and electronic cigarettes on qualitative and quantitative saliva parameters

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Abstract

The available literature indicates that smoking causes quantitative and qualitative changes in saliva. However, there is a lack of studies summarizing the knowledge in this area, and there are no clear guidelines on the use of salivary biomarkers for assessing exposure to cigarette smoke (CS). The present work aimed to provide a systematic review of the literature regarding the influence of smoking traditional and electronic cigarettes, as well as heat-not-burn products, on salivary homeostasis. An electronic search of the literature from 1982 to 2023 was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Based on the inclusion criteria, 65 studies were used for the final review. Smoking traditional as well as electronic cigarettes negatively affects salivary biomarkers, including the salivary flow rate, pH, antibody titer, electrolyte concentration, microflora composition, redox balance, and inflammation, in terms of both quantity and quality. However, to date, only single salivary biomarkers have been compared in traditional and electronic cigarette smokers. It can be concluded that the salivary production rate, pH, microbiome, and cytokines can be used to assess exposure to CS smoke. There is a lack of convincing evidence to compare the toxic influence of traditional and electronic cigarettes on salivary homeostasis. Future experiments should include long-term randomized clinical trials on larger populations of smokers.

Keywords: smoking, saliva, biomarker

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Introduction

Despite the implementation and popularization of anti-tobacco programs, addiction to smoking continues to be a global public health problem[1] and is one of the primary causes of premature deaths, decreasing life expectancy by up to 8 years. Cigarette smoke (CS) contains over 5,000 harmful substances, 400 of which are scientifically proven carcinogens, such as formaldehyde, benzene and vinyl chloride,[2] with the latest research demonstrating that the poisonous chemicals contained in cigarettes reach every organ in the human body.[3] In addition to an increased risk of cancer, smoking raises the probability of stroke, respiratory diseases and inflammation, and weakens immune functions.[4,5] Smoking also adversely affects the oral ecosystem, the consequences of which are well-documented; they comprise relatively mild complaints (e.g., bad breath, tooth discoloration, and an increased accumulation of plaque and tartar), but also life-threatening diseases (including oral cancer).[6,7]

The first protective barrier against the chemicals contained in cigarettes is saliva, which, under normal conditions, constantly moistens the mucous membrane and the teeth.[8] Saliva is secreted from large and small salivary glands, and serves numerous functions in the body, including inhibiting the development of bacteria, and preventing the demineralization of hard tissues in the oral cavity and the formation of carious and non-carious cavities.[9,10] Furthermore, saliva is a source of enzymatic and non-enzymatic antioxidants, preventing redox homeostasis disorders in the oral cavity. It also enables the formation of a bolus, and initiates sugar and fat digestion. Reduced saliva secretion is a serious health problem leading to diseases within the oral cavity. It has been demonstrated that salivary homeostasis is influenced by various factors, including chronic diseases, such as obesity, insulin resistance, chronic kidney disease, congestive heart failure, psoriasis, stroke, and neurodegenerative diseases (Alzheimer's disease and dementia).[11,12]

Numerous studies have reported the negative impact of smoking on the salivary glands.[13–15] Therefore, the assessment of salivary biomarkers may have a diagnostic value in monitoring the oral health consequences of smoking. Indeed, changes in the quantitative and qualitative composition of smokers' saliva have been reported. However, no papers have summarized the knowledge in this area, and there are no clear guidelines on the use of salivary biomarkers for assessing exposure to CS smoke.

A "healthier" alternative to traditional cigarettes are electronic cigarettes (e-cigarettes; ECs),[16] mechanical devices that heat special inhalation solutions and provide the user with an experience similar to traditional smoking.[17,18] E-cigarette liquid mainly consists of propylene glycol, glycerol, aromas, and nicotine,[19,20] although more detailed tests have confirmed the presence of formaldehyde, acrolein and heavy metals.[21,22] Although the common belief in a reduced harmfulness of ECs is now becoming increasingly controversial, the use of ECs, or so-called "vaping", has become extremely popular among young people.[23,24] Aggressive marketing campaigns have also led to the "renormalization" of smoking,[24] with the U.S. Food and Drug Administration (FDA) reporting a 900% increase in the use of ECs among high school students.

E-cigarettes were introduced onto the market and patented in China in 2006.[25] Due to their relatively short presence on the consumer market as compared to traditional cigarettes, the long-term effects of ECs are unknown and the knowledge about their short-term effects is still very limited. It is acknowledged that direct exposure to the EC aerosol may impair blood vessel and respiratory functions, but there is still a substantial gap in the knowledge on the effects of vaping on salivary homeostasis.[26,27] Also, there have been no reviews evaluating the influence of traditional cigarettes and the abovementioned new-era devices on various salivary parameters. Taking into consideration the harmful effects of smoking on the oral ecosystem, as well as the lack of non-invasive biomarkers to assess exposure to smoke, we conducted a systematic review of the literature on the salivary biomarkers in the smokers of traditional cigarettes, ECs and heat-not-burn products. We are the first to compare the available literature regarding the effects of smoking traditional and electronic cigarettes on the salivary secretion and composition.

Material and methods

Search strategy

The literature search was conducted up to September 3, 2023, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines,[28] using the PubMed/MEDLINE, Scopus and Web of Science databases. We only evaluated international publications written in English. The available literature was browsed based on the following keywords: 'cigarette and oral health'; 'cigarette and saliva'; 'smoking and saliva'; 'smoking and salivary flow rate'; 'smoking and salivary pH'; 'smoking and salivary oxidative stress'; 'smoking and oral microbiome'; 'smoking and salivary immunoglobulins'; 'smoking and oral inflammation'; 'smoking and salivary minerals'; 'e-cigarette and saliva'; 'electronic cigarette and saliva'; 'vaping and saliva'; 'heated tobacco and oral health'; and 'heated tobacco and saliva'. The inclusion and exclusion criteria are presented in Table 1.

Table 1. Inclusion and exclusion criteria of the systematic review

Inclusion criteria	Exclusion criteria
– articles written in English only – publications on the influence of smoking traditional and/or electronic cigarettes on quantitative and qualitative changes in salivary parameters, including the rate of saliva secretion by the salivary glands, the salivary pH and buffer capacity, the concentrations of antibodies and inflammatory mediators, and the mineral content, as well as the effect on the salivary microbiome and antioxidant barrier – research with the participation of humans as well as experimental works, including in vitro tests – clinical trials conducted on a group of at least 20 people – meta-analyses	– publications written in a language other than English – publications assessing the influence of smoking on the concentrations of cotinine, thiocyanates and inflammatory mediators, the mineral content and the antioxidant barrier in serum/urine only, excluding saliva – research methodology comprising only smokers with systemic diseases – clinical studies performed on a group of fewer than 20 people – surveys – case descriptions

Data extraction

Data pre-selection was carried out independently by 2 authors, based on the assessment of the titles and abstracts of the manuscripts, followed by a thorough review of the texts of the selected articles. Publications that met the inclusion criteria were considered for the review. In cases of doubt over the content of an article, the other authors were consulted. The reliability level of the researchers was determined using Cohen's kappa coefficient (κ), which amounted to 0.92. All publications were evaluated in terms of methodology to ensure data quality, and the following variables were recorded: author(s); year of publication; study design; size of the study population; inclusion and exclusion criteria; duration of the study; and research results.

Results and discussion

The literature search revealed 34,745 works from the PubMed/MEDLINE, Scopus and Web of Science databases, of which 32,462 were removed due to duplication. A total of 2,283 abstracts were read, with 218 meeting the inclusion criteria. Among the qualified articles, 153 either were irrelevant to the subject of our review or presented other type of paper than required, leaving 65 papers included. A PRISMA flow diagram presenting the search strategy is shown in Fig. 1.

According to the Oxford Center for Evidence-Based Medicine (CEBM) 5-level classification scale of diagnosis, most of the studies presented the 3rd or 4th level of evidence (clinical control studies).[29] Only 3 studies used a prospective cohort design.

Table S1 (the supplementary material is available on request from the corresponding author) shows the

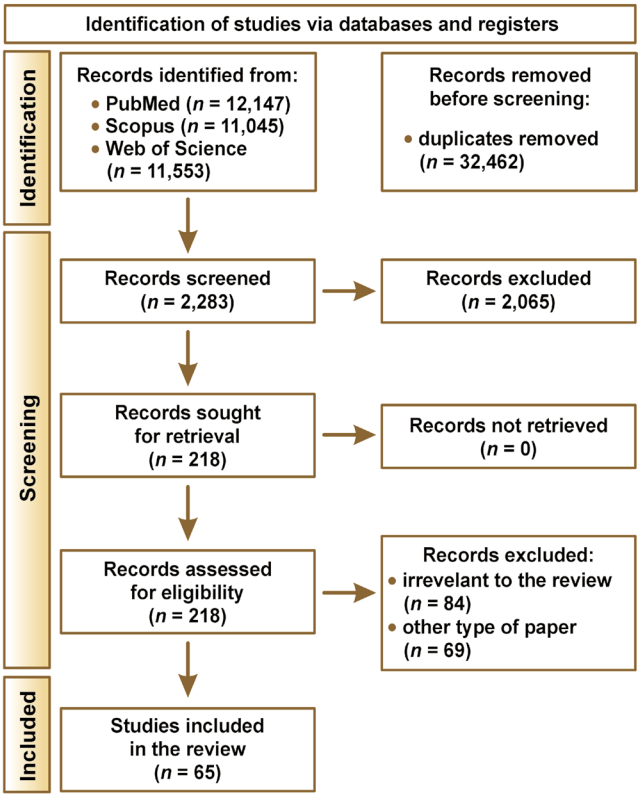

Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram presenting the search strategy

summarized quality assessment according to the Study Quality Assessment Tool guidelines issued by the National Heart, Lung and Blood Institute (NHLBI) within the U.S. National Institutes of Health (NIH).[30] The critical assessment was performed by summing the points for each criterion with regard to the potential risk of bias (1 – low; 0.5 – indeterminate; and 0 – high). The most commonly encountered risks of bias were sample size justification, randomization and the blinding status of participants. Forty-seven studies (72.3%) were classified as having "good" quality (≥80% of total points), and 18 (27.7%) were classified as having "intermediate" quality (≥60% and <80% of total points).

The vast majority of studies included in the review focused on traditional cigarettes. Only 10 compared the toxicity of traditional vs. electronic cigarettes, using salivary biomarkers. The methodology and endpoints of all studies reviewed are presented in Tables S2–S7 in the supplementary material (available on request from the corresponding author).

The multi-directional effect of smoking on various salivary parameters has been widely documented, and includes a decrease in the salivary flow rate (SFR) and pH, a reduction in the mineral content, and changes in microflora composition, and concentrations of antibodies, antioxidants and inflammatory mediators (Fig. 2). A summary of the results of the systematic review is presented in Table 2.

Table 2. Summary of the studies included in the systematic review. The table shows changes in the saliva of smokers as compared to controls (non-smokers)

Saliva characteristics			Cigarette smokers		Electronic cigarette smokers	
			unstimulated saliva	stimulated saliva	unstimulated saliva	stimulated saliva
Flow rate, viscosity and buffer capacity of saliva	flow rate	↑	–	–	–	–
		↓	33, 34, 35, 36, 37, 38, 39, 40	39	–	–
		≈	45, 46, 53	45, 53	142, 147	
	viscosity	↑	34	–	–	–
		↓	39	39	–	–
	pH	↑	–	–	–	–
		↓	34, 35, 36, 37, 151	–	147	–
		≈	39, 46	39	148	–
Salivary mineral content	Na^+	↑	–	–	–	–
		↓	68	–	–	–
		≈	69	64	–	–
	K^+	↑	–	–	–	–
		↓	68	–	–	–
		≈	69	64	–	–
	Ca^{2+}	↑	69	67	148	–
		↓	63	64, 66	–	–
		≈	65, 69, 151	–	–	–
	Mg^{2+}	↑	63	67	–	–
		↓	68	–	–	–
		≈	69	64	–	–
	Zn^{2+}	↑	–	–	–	–
		↓	–	64	–	–
		≈	–	–	–	–
	Pb^{2+}	↑	–	–	–	–
		↓	–	64	–	–
		≈	–	–	–	–
	PO_4^{3-}	↑	151	67	–	–
		↓	63, 69	64	–	–
		≈	–	66	148	–
Salivary inflammation and immune response	TNF-α	↑	–	–	–	–
		↓	–	–	–	–
		≈	–	–	–	–
	INF-γ	↑	141	–	–	–
		↓	–	–	–	–
		≈	–	–	–	–
	IL-1β	↑	142, 143	–	150, 155	–
		↓	–	–	–	–
		≈	–	–	142	–
	IL-2	↑	86, 141	–	–	–
		↓	–	–	–	–
		≈	–	–	–	–
	IL-4	↑	86	–	–	–
		↓	–	–	–	–
		≈	–	–	–	–
	IL-5	↑	–	–	–	–
		↓	86	–	–	–
		≈	–	–	–	–

Saliva characteristics			Cigarette smokers		Electronic cigarette smokers	
			unstimulated saliva	stimulated saliva	unstimulated saliva	stimulated saliva
Salivary inflammation and immune response	IL-6	↑	142	–	150	–
		↓	–	–	–	–
		≈	–	–	142	–
	IL-7	↑	86	–	–	–
		↓	–	–	–	–
		≈	–	–	–	–
	IL-8	↑	–	–	–	–
		↓	143	–	–	–
		≈	–	–	–	–
	IL-10	↑	–	–	–	–
		↓	86	–	–	–
		≈	–	–	–	–
	IgA	↑	98, 107	107	–	–
		↓	101, 102, 103, 104, 107	108	–	–
		≈	99, 100, 105	100, 106	151	–
	IgG	↑	–	–	–	–
		↓	98	–	–	–
		≈	–	–	–	–
	IgM	↑	–	–	–	–
		↓	98	–	–	–
		≈	–	–	–	–
Salivary antioxidant barrier	TAC	↑	–	–	–	–
		↓	127, 128, 130	129	152	–
		≈	126, 134	–	–	–
	CAT	↑	–	–	–	–
		↓	120, 121	–	–	–
		≈	–	–	–	–
	SOD	↑	119, 134	–	–	–
		↓	117, 118, 124	–	–	–
		≈	–	–	–	–
	Px	↑	117, 118	–	–	–
		↓	122, 123, 134	–	–	–
		≈	–	–	–	–
	UA	↑	–	–	152	–
		↓	127	–	–	–
		≈	118, 126	–	–	–
	GSH	↑	126	–	–	–
		↓	–	–	–	–
		≈	–	–	–	–
	vitamin C	↑	–	–	–	–
		↓	121	–	–	–
		≈	–	–	–	–

Na^+ – sodium ion; K^+ – potassium ion; Ca^{2+} – calcium ion; Mg^{2+} – magnesium ion, Zn^{2+} – zinc ion; Pb^{2+} – lead ion, PO_4^{3-} – phosphate ion; TNF-α – tumor necrosis factor alpha; INF-γ – interferon gamma; IL – interleukin; Ig – immunoglobulin; TAC – total antioxidant capacity; CAT – catalase; SOD – superoxide dismutase; Px – peroxidase; UA – uric acid; GSH – glutathione; ↑ statistically significant increase; ↓ statistically significant decrease; ≈ no statistical difference. The numbers indicate specific literature items from the reference list.

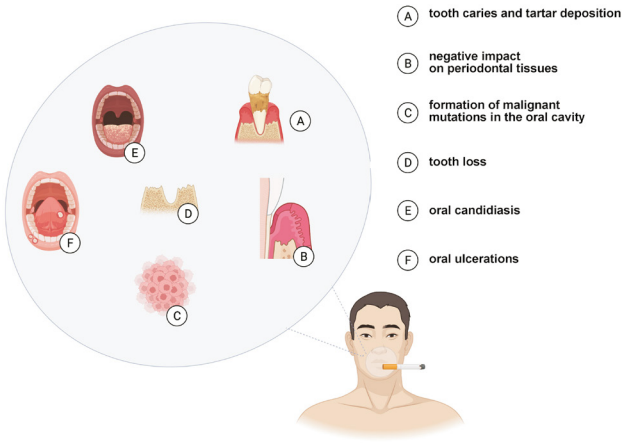

Fig. 2. Influence of smoking on various properties of saliva

Effect of cigarette smoking on the flow rate, viscosity and buffer capacity of saliva

The constant moistening of the oral cavity by saliva determines dental and periodontal health, with a proper SFR being a prerequisite for the continued remineralization of teeth, soft tissue protection and regeneration, antimicrobial effect of saliva, and the initial digestion of sugars contained in food.[31,32] Interestingly, smoking has been proven to reduce SFR. Confirming the previous observations,[33–40] Singh et al. demonstrated a significant decrease in SFR in cigarette smokers.[36] One of the mechanisms leading to a reduced SFR may be alterations in the tissue architecture of the salivary glands.[41] In rats exposed to tobacco smoke, atrophic changes and inflammatory infiltration within acinar cells were observed in the parotid and submandibular glands.[41] In secretory cells, the extracellular matrix is enlarged and fibrotic due to an increase in type III and type I collagen fibers. Meanwhile, structural alterations in glandular cells disturb the microenvironment of the salivary gland stroma and may be responsible for reduced salivary secretion. Increased type I collagen density can also be observed in the stroma of malignant neoplasms.[42] A reduced SFR induces symptoms such as dry mouth (including difficulty in swallowing and speaking), impaired taste perception, and sore tongue and lips (which become dry and cracked), contributing to increased thirst and discomfort, and may also explain an increased incidence of dental caries, periodontal disease and fungal infections in smokers.[43,44]

Several authors also reported increased saliva secretion after the exposure of the oral mucosa to CS.[45,46] An increased SFR in smokers may be caused by the presence of nicotinic receptor agonists (nicotine and cytosine) in cigarettes, triggering the chemical stimulation of taste receptors and stimulating the salivary glands.[47,48] In addition, nicotinic agonists lead to an increased release of norepinephrine and acetylcholine from autonomic nerve endings via the activation of the nicotinic receptor subtype α3β4.[48] Those who have started smoking recently may experience a temporary increase in salivary gland activity in response to the stimulation of the nerve endings of taste receptors by alkaloids contained in tobacco smoke, followed by a gradual quieting of the receptors.[49] Another explanation for increased salivation in smokers could be found in the "irritation" theory.[50] Smoking can significantly increase salivation by the parotid glands as compared to the state before smoking due to the exposure of the oral mucosa to the toxic components of CS (irrespective of the smell), inducing the secretion of stimulated saliva.[51] Responding to irritation by increasing saliva production is consistent with its protective function. To protect the oral mucosa, toxic and damaging factors are diluted with an increased amount of saliva, and rinsed. Although smoking is considered pleasant for smokers and unpleasant for non-smokers, the response to irritant stimuli should be greater in non-smokers.[51,52]

In contrast, Petrušić et al. found no significant difference in the amount of unstimulated and stimulated saliva between smokers and non-smokers (though SFR was lower in smokers).[53] The authors concluded that SFR in smokers decreased with smoking duration, and the amount of the secreted saliva did not depend on the number of cigarettes smoked per day. They also observed a change in the quality of salivary gland secretion.[53]

In the majority of nicotine addicts, saliva is reported to be thick and viscous, while in non-smokers, it is thinner.[34,39] In a study by Kusumaningrum et al., 57% of smokers demonstrated moderate saliva viscosity, 43% were included in the poor saliva viscosity category, and no participants demonstrated normal saliva viscosity.[34] In the group of non-smokers, 75% of participants had normal saliva viscosity, and 25% had moderate saliva viscosity, indicating that no subjects had poor saliva viscosity.[34] An increase in saliva viscosity due to smoking may be connected with a high sensitivity of the parotid glands to tobacco smoke toxins.[54,55] The parotid glands are responsible for producing watery, serous saliva, and its absence is compensated by the submandibular and sublingual salivary glands, which secrete thicker saliva with a high mucin content.[56,57] Thick saliva does not effectively moisten the oral cavity, which can cause symptoms similar to those of decreased saliva secretion, and lead to a subjective feeling of dryness in the mouth and intensified symptoms of dental caries.[58] Furthermore, people with abnormal saliva density often complain about impaired swallowing and difficulty in forming a food bolus.

In addition to the proper flow rate and viscosity of saliva, an important factor in maintaining healthy teeth and periodontium is its buffer capacity.[59] The stimulated saliva secreted by the parotid glands contains significant amounts of bicarbonate buffers that maintain a normal pH, and neutralize acids from food and those produced by cariogenic bacteria.[60,61] Factors that reduce SFR also decrease the buffer capacity of saliva and increase the risk of caries. Indeed, patients with a low SFR had lower bicar-

bonate concentrations, and this relationship correlated with an increased prevalence of caries and cavities of non-carious origin.[34,39] In a study by Singh et al., the mean salivary pH reached 6.30 ±0.36 in smokers and 7.10 ±0.24 in non-smokers.[36] After measuring the salivary pH of smokers, Saputri et al. found that 67.5% of them had pH < 6.7, for 32.5%, it ranged from 6.7 to 7.4, while pH > 7.4 was not recorded in any participant.[35] Voelker et al. showed no association between the nicotine levels in cigarettes and the pH of saliva.[39] In both studies, a decrease was observed in the pH values along with lower values of SFR.[35,39] Furthermore, it was proven that the saliva buffering response as a consequence of drinking acidic carbonated beverages was 20% lower in smokers than in non-smokers (Tables S2–S4, available on request from the corresponding author).

Effect of smoking on the salivary mineral content

Saliva contains a number of electrolytes, the final content of which is determined through a process that takes places at the level of the striated ducts of the salivary glands.[62] Although there is little data on the relationship between oral health and ions present in saliva, the salivary electrolyte levels are determined by general health.[63–69] Calcium (Ca) and phosphorus (P) ions, in addition to participating in the remineralization of dental hard tissues (along with sodium (Na) and magnesium (Mg) ions), contribute to plaque mineralization and tartar formation.[70–73] A similar function is performed by fluorine (F), which binds to the amino groups in hydroxyapatite and replaces hydroxide ions to form fluorapatite, which is more resistant to acids produced by bacteria and contained in food.[74] A few authors attempted to determine the salivary electrolyte profile of smokers, with most studies indicating no effect or an insignificant decrease in the electrolyte content. Kolte et al. investigated the relationship between smoking and the salivary levels of Ca, Mg and P, and found decreased concentrations in the smoking group as compared to non-smoking controls.[63] Similarly, Sewón et al. revealed a significantly decreased Ca content in the saliva collected from 90 smokers.[66] Changes in the salivary concentrations of Ca, Mg and phosphate ions, along with decreased plaque pH, may be responsible for impaired enamel mineralization, which facilitates tartar accumulation in smokers. Other studies reported a decreased salivary concentration of zinc (Zn), serving as a cofactor for superoxide dismutase (SOD).[64,75] Superoxide dismutase is a vital component of the body's antioxidant mechanisms responsible for catalyzing the dismutation reaction of superoxide radicals to hydrogen peroxide and oxygen. Thus, a decrease in the salivary Zn concentration may partially explain reduced SOD activity and increased oxidative stress in smokers (Table S5, available on request from the corresponding author).

Effect of smoking on oral microflora composition

Several studies demonstrated that smokers were less diligent in their oral hygiene regime than non-smokers.[76–78] The level of oral hygiene is determined by the number of caries-related bacteria, including variable streptococci and lactic acid bacteria, the levels of which can be measured in saliva.[79–82]

In a study by Heintze, smokers had a significantly higher number of lactobacilli and *Streptococcus mutans*, considered major cariogenic bacteria.[83] The count of lactobacilli in saliva correlated positively with the number of cigarettes smoked per day, and about 40% of smokers had the count of *S. mutans* more than doubled as compared to non-smokers.[83] Saliva plays a critical role in preventing bacteria from adhering to tissues. Low pH and flow rate of saliva, as well as its low buffer capacity, may additionally favor the development of lactic acid bacilli. In addition, it was observed that smoking correlated with the number of commensal *Candida albicans*.[84] The study indicated that smoking traditional cigarettes inhibited the growth of Gram-positive cocci, which delay the colonization of carious bacteria, including the pioneer species of *Neisseria*, and CS promoted the growth of Gram-negative microorganisms.[85–87] On the other hand, Nakonieczna-Rudnicka and Bachanek reported no significant relationship between the number of *S. mutans* and lactobacilli in saliva and smoking duration or the number of cigarettes smoked per day.[88]

In the past, it was believed that the sugar content in cigarettes was the main contributor to the growth of caries-related bacteria, and that the chemical compounds contained in cigarettes did not play a substantial role. However, the current prevailing assumption is that nicotine stimulates the development of cariogenic microflora.[79] Nicotine is responsible for an increased adhesion of planktonic bacteria to the tooth biofilm and its thickening due to an increase in the synthesis of extracellular polysaccharides (EPS), glucosyltransferase (Gtf) and glucan-binding protein (Gbp) at the mRNA and protein levels.[89,90] The influence of nicotine on the activity of lactate dehydrogenase (LDH) was also assessed.[91] Lactate dehydrogenase is an enzyme that catalyzes the last step in the bacterial glycolytic pathway, converting pyruvate to lactate.[92] Although nicotine does not directly affect LDH, it indirectly enhances its activity by increasing the total amount of bacteria.[90] As such, more lactic acid may contribute to the risk of caries (Table S6, available on request from the corresponding author).

Effect of smoking on the salivary immune response

Saliva has a defensive function, since it contains several immunoglobulins (Ig) – IgA, IgG, IgM – and antioxidant

factors (lysozymes).[93,94] Secretory IgA (SIgA) is the predominant immunoglobulin in salivary gland secretions,[95] and is the first line of the host defense against pathogens that colonize or attack the surfaces covered by external secretions.[94,96,97] The primary function of SIgA is to limit microbial adherence and the penetration of foreign antigens into the mucosa. Naturally occurring SIgA antibodies have been detected in saliva. Although the role of SIgA in the colonization and regulation of the native bacterial flora is still questionable, less abundant salivary IgG and IgM limit bacterial adherence to enamel and cheeks.[94] The effect of smoking on the levels of salivary immunoglobulins has been widely documented,[98–109] with most studies reporting a significant reduction in the SIgA content under the influence of smoking. In the work of Giuca et al., the concentrations of IgA, IgG and IgM antibodies decreased in smokers by 91%, 99% and 83%, respectively, as compared to the non-smoking control group.[101] On the other hand, Tarbiah et al.[98] and Engström and Engström[107] observed an increased IgA antibody concentration in the saliva of smokers.[98,107] In contrast, Olayanju et al. revealed a decreased IgM content, with no effect on the amount of IgA and IgG.[105] Abnormal salivary immunoglobulin levels may partially explain an increased incidence of periodontal disease in smokers. Both the local irritant effects of tobacco smoke and exposure to nicotine and its metabolites can disrupt humoral immune mechanisms in the oral cavity. In smokers, there are abnormalities in the response to the antigens of plaque bacteria, involving impaired chemotaxis, phagocytosis and neutralization of bacterial enzymes and toxins.[97,110] Of particular importance is a decrease in salivary IgG, involved in the activation of the complement system and the removal of *Aggregatibacter actinomycetemcomitans*, responsible for periodontitis progression.[107]

Nagler determined the salivary immunological profile in compulsive smokers and its potential correlation with the development of oral cancer.[2] Biochemical and immunological analyses showed a significant reduction in the IgG antibody level, as well as increased albumin content and activity of amylase, LDH, matrix metalloproteinase 2 (MMP-2), and MMP-9 by 61%, 86%, 65%, 35%, and 55%, respectively. Matrix metalloproteinases are matrix-degrading enzymes secreted during the migration of epithelial cells to the underlying connective tissue, with MMP-2 and MMP-3 shown to play a central role in oral cancer invasion and metastasis.[2] Therefore, there is an imbalance between oral protective and procarcinogenic factors in smokers. Studies suggest an increased rate of epithelial cell exfoliation into the oral cavity, causing many compounds to pass from serum into saliva through the compromised oral and gingival mucosa.[108]

Although the exact mechanisms of the influence of CS on the body are still unknown, adverse reactions to the cellular immune response are associated with exposure to aromatic compounds, heavy metals, nicotine, and other toxins.[50,111] Indeed, long-term exposure to nicotine has been shown to suppress the cellular response through reduced antibody production, disrupted antigenic signaling in T lymphocytes and T cell anergy[112] (Table S6, available on request from the corresponding author).

Effect of smoking on the salivary redox balance

Cigarette smoke is a mixture of thousands of substances responsible for generating reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as benzene, 2-napthylamine, cadmium (Cd), and benzopyrene. Free radicals are highly reactive species that readily oxidize cellular biomolecules (i.e., lipids, proteins and nucleic acids).[113,114] Situations in which antioxidant mechanisms are unable to cope with the neutralization of ROS/RNS lead to oxidative stress,[115] which disrupts cell metabolism, and ultimately leads to cell death by apoptosis or necrosis.[116] Saliva is the first biological fluid whose antioxidant systems oppose the free radicals produced while smoking. Several studies have indicated that smoking affects the antioxidant barrier of saliva.[41] Fujinami et al. showed that passive exposure to tobacco smoke resulted in a significant reduction in the activity of salivary peroxidase (Px), catalase (CAT) and α-amylase.[41] Interestingly, changes in salivary antioxidant enzymes were accompanied by histological changes within the salivary glands. After 30 days of the experiment, the researchers observed an increase in the interlobular duct area, part of the striated duct, and vacuolar degeneration in the entire CS-exposed parotid gland, as well as in the central part of the submandibular gland. The salivary glands are highly sensitive to oxidative cellular damage, and since oxidative stress is a key factor responsible for impaired secretory function of the salivary glands, hyposalivation in smokers may be caused by disturbances in redox homeostasis.[41] Nevertheless, this hypothesis requires further investigation.

Depletion of antioxidant enzymes has also been observed in clinical trials, with many studies clearly showing that cigarette smoking is accompanied by a decrease in the activity of SOD, CAT and Px (the latter by up to 76%).[41,117–123] Enzymatic antioxidants are the first line of defense, preventing the reaction of free radicals and their derivatives with salivary biomolecules. When the activity of Px and CAT is reduced, intoxication with hydrogen peroxide in the oral cavity is significantly reduced.[120–125] In the presence of transition metal ions, hydrogen peroxide initiates the Fenton and Haber–Weiss reactions, leading to the formation of a highly reactive hydroxyl radical (•OH).[116] The reactive oxygen species generated during smoking are also responsible for a decrease in the concentrations of reduced glutathione (GSH) (probably as a result of combining cysteine contained in GSH with aldehydes, which are components of CS), as well as uric acid (UA) and LDH.[126,127] Uric acid the major

antioxidant of saliva, determining up to 70–80% of its antioxidant capacity. Some other low-molecular-weight hydrophilic antioxidants, such as vitamin C, also play a key role in counteracting salivary oxidative stress. Smokers have significantly lower levels of salivary vitamin C.[128] The reason for the abovementioned phenomenon is multifactorial. Firstly, smokers consume less dietary antioxidants and absorb them less efficiently than non-smokers. Secondly, the protective effect of antioxidants is more rapidly worn down in smokers by continuous exposure to ROS.[128] Therefore, it is not surprising that in people exposed to CS, a significant decrease in salivary total antioxidant capacity (TAC) is observed.[126,128–131]

Salivary TAC characterizes the resultant ability of saliva to scavenge oxygen free radicals. It is well known that the evaluation of salivary TAC provides much more information than the assessment of individual antioxidants separately, as the synergistic effects of ROS scavengers are often observed.[59] Bakhtiari et al. demonstrated a 29% drop in TAC in smokers as compared to the control group.[128] In another study, Nagler et al. showed that salivary TAC (the ImAnOx® assay) and SOD activity were decreased in smokers by 32% and 12%, respectively.[2] A consequence of a diminished antioxidant barrier is enhanced oxidation of salivary lipids, proteins and nucleic acids. In a study by Demirtaş et al., the concentration of malondialdehyde (MDA) in the saliva of smokers was significantly higher as compared to the control group and the group of passive smokers,[132] which partially agreed with the results of other research groups. Evidence showed that salivary MDA has a pathological role in multi-step oral carcinogenesis and cancer progression. Metgud and Bajaj observed a significant elevation in the salivary MDA levels, which progressed from a healthy control group, through a precancerous state, to individuals with squamous cell carcinoma (SCC),[133] while Nagler et al. showed higher salivary total protein carbonyls (by 126%) in the smoking group as compared to non-smokers.[2] Reznick et al. also noted significantly increased carbonylation of salivary proteins in heavy smokers and non-smokers exposed to cigarette smoking one time.[123]

On the other hand, Kanehira et al.[134] and Baharvarnd et al.[119] reported increased SOD activity in the saliva of cigarette smokers. It can be speculated that the enhancement of the salivary antioxidant barrier is an initial adaptive response to CS-induced ROS overproduction in novice smokers. With an increasing duration of smoking, the antioxidant reserves of saliva may become depleted and the oxidative injury to saliva biomolecules increases. Further research is needed on the relationship between smoking duration, the number and type of cigarettes smoked and salivary redox homeostasis.

Interestingly, CS-induced oxidative stress does not decrease after the oral administration of antioxidant compounds, including a strong ROS-scavenger vitamin C.[135] Even after 4 weeks of vitamin C supplementation, smokers did not achieve an increase in salivary TAC to the levels observed in the control group.[135] Azimi et al. investigated the effect of a 3-week consumption of green tea on the salivary antioxidant barrier in heavy smokers, occasional smokers and non-smokers.[136] At the beginning of the experiment, the antioxidant capacity of saliva differed between the 3 groups, reaching the highest values in the control group and the lowest in the heavy smokers group. After 7 days of green tea consumption, there was no significant difference between occasional smokers and non-smokers. Heavy smokers were characterized by a significantly higher TAC as compared to the other 2 groups, with the same relationship occurring after 14 and 21 days of the study. The TAC level showed an upward trend during the experiment, and the antioxidant capacity on the 21st day of green tea drinking was significantly higher as compared to the measurement made at baseline. Nevertheless, the TAC values in the smoking group did not come close to those in the control group. The beneficial effect of green tea may be explained by a high content of catechins, monomeric polyphenols that have a strong antioxidant effect, supporting ROS sweeping. Interestingly, early studies showed that green tea catechins could be detected in saliva even after a vigorous rinsing of the mouth[136] (Table S7, available on request from the corresponding author).

Effect of smoking on the salivary inflammation

Salivary redox homeostasis is inextricably linked to oral inflammation, with smoking shown to interfere with the synthesis and secretion of inflammatory mediators in saliva. Cigarette smoke increases the release of tumor necrosis factor alpha (TNF-α), interleukins (IL) IL-1, IL-6 and IL-8, and granulocyte–macrophage colony-stimulating factor (GM-CSF).[137,138] The main source of inflammation in the mouth comes from activated macrophages, which contribute to the elimination of bacteria and viruses through phagocytic and cytotoxic activity. Their activation results in increased production of proteolytic enzymes, cytokines, chemokines, growth factors, and oxygen free radicals.[137] However, macrophages also show high reactivity to CS. In smokers, macrophages produce more TNF-α, IL-1 and IL-6 than in non-smokers.[139] In addition to changes in macrophage morphology (e.g., cytoplasm thickening and its higher density), these cells also show higher CD14 expression, stronger inhibition of lymphocyte and NK (natural killer) proliferation, and less ability to phagocytose microorganisms. Cigarette smoke also changes the T helper (Th)1/Th2 cell ratio.[140]

Exposure to CS generally reduces the ability of the circulating dendritic cells to present antigens to lymphocytes. Rahimi et al. found a significant increase in the levels of interferon gamma (IFN-γ) and IL-2 in the saliva of smokers as compared to non-smokers.[141] Thus, CS

mainly affects the balance of cytokines produced by Th cells. Importantly, the concentrations of these cytokines increased with smoking duration.[141] Mokeem et al. demonstrated significantly higher levels of pro-inflammatory IL-1β and IL-6 in the saliva of cigarette smokers.[142] It is worth mentioning that IL-1β in saliva is associated with active smoking, regardless of the number of cigarettes smoked, in contrast to the level of TNF-α, the concentration of which positively correlates with the number of cigarettes smoked daily. In a study by Nishida et al., the analysis of salivary cytokines in workers aged 18–62 years showed that the mean level of IL-1β in saliva was significantly higher in passive smokers (190 pg/mL) than in non-smokers (164 pg/mL); however, there was no difference between passive and active smokers (167 pg/mL).[143] Moreover, the levels of other biomarkers, such as prostaglandin E2 (PGE2), MMP-9, lactoferrin, albumin, and aspartate aminotransferase (AST), were significantly lower in active smokers.[143]

Nicotine, hydroquinone and carbon monoxide are the main toxins responsible for the immunosuppressive and pro-inflammatory effects of CS. Nevertheless, nicotine can also reduce IL-6 production. Rodríguez-Rabassa et al. observed significant differences in the expression of salivary interleukins (↑ IL-2, ↑ IL-4, ↓ IL-5, ↓ IL-10), adrenocorticotropic hormone (ACTH) (↑), insulin (↓), and leptin (↓) in smokers as compared to non-smokers.[86] The effects of smoking on salivary cytokines and chemokines are extensively studied in relation to periodontitis. There are a few reports on the concentrations of salivary cytokines in the context of smoking and the immune response of healthy participants. For example, in a study by Rathnayake et al., a group of healthy smokers showed a significantly reduced salivary IL-8 concentration as compared to non-smoking controls.[144] Based on these data, it can be concluded that smoking has the potential to suppress the host defense system and promote the progression of periodontal disease. The effect of smoking on salivary cytokines is likely to vary with regard to age, the characteristics of the host defense system and periodontitis progression.[144] Ageing is characterized by quantitative and qualitative changes in the immune system (i.e., increased levels of pro-inflammatory cytokines and decreased levels of anti-inflammatory cytokines). Unfortunately, the exact mechanisms through which tobacco smoke affects oral homeostasis have not yet been thoroughly investigated (Table S6, available on request from the corresponding author).

Effect of smoking electronic cigarettes on the salivary secretion and composition

The effect of EC smoking on saliva remains unclear.[145] The first smokeless cigarette was patented by Herbert A. Gilbert in the state of Pennsylvania, USA, in 1967. However, the peak of EC popularity was in 2004, when Hon Lik, a Chinese pharmacist, modernized the original version of ECs and started distributing them in his local market.[146] Undoubtedly, due to the short presence of ECs on the consumer market as compared to tobacco cigarettes, their long-term effects on human health cannot be determined. There are also scarce literature sources available on the effects of vaping on the secretion of saliva. Therefore, further research is necessary to assess the impact of EC use.

Several publications have evaluated the salivary pH and flow rate in EC smokers. Lestari et al. investigated the direct effect of smoking ECs on SFR and the salivary pH, and their results clearly indicated a significantly lower pH in EC smokers as compared to non-smokers, but no significant difference in the SFR levels between the 2 groups.[147] However, the methodology of their experiment did not include the collection of saliva from traditional cigarette smokers and the comparison of the traditional smokers with the vaping group.[147] This relationship was taken into account by Mokeem et al., whose results did not reveal any changes in the SFR values between the 3 study groups (EC smokers vs. traditional smokers vs. non-smokers).[142]

In research performed by Cichońska et al., pH was lower, and total protein, Ca and phosphate concentrations were higher in EC users in than non-smokers.[148] The saliva of EC users showed changes in its physicochemical composition as compared to traditional smokers and non-smokers, but significant differences were only observed for the Ca concentration.[148]

An imbalance in oral microbiota was suggested in EC smokers.[149,150] To measure the effect of vaping on oral microbiota, a team of researchers assigned around 100 volunteers into one of the 3 groups – traditional smokers (an average of half a pack a day), EC smokers (half an EC a day) and non-smokers.[150] The first finding was that the rates of periodontal disease severity (periodontal pocket depth (PPD) and bleeding on probing (BOP)) among EC smokers were significantly lower (42.5%) than in traditional smokers (72.5%), but were considerably higher as compared to non-smokers (28.2%). The second conclusion was that EC smokers showed a dysbiosis of oral microbiota comparable to that caused by traditional smoking. Their saliva was generally richer in bacteria as compared to non-smokers and showed the proliferation of various species harmful to oral health. Furthermore, human cells exposed to the EC aerosol showed increased susceptibility to bacterial infections as compared to cells exposed to clean air. The results of this study (conducted on humans (in vivo) and cells (in vitro)) confirm that vaporization disturbs the balance of oral microbiota and increases susceptibility to infections.[150] Cichońska et al. investigated changes in the antibacterial properties of the saliva of EC smokers.[151] A total of 120 subjects (40 EC smokers, 40 traditional smokers and 40 non-smokers) where recruited to the

experiment which included the assessment of the lysozyme, lactoferrin and IgA content in saliva. The study revealed that EC users had lower salivary lysozyme and lactoferrin levels as compared to non-smokers, although no difference in the IgA antibody concentration was observed between the 2 groups. The results clearly demonstrated that ECs decrease the antimicrobial capacity of saliva. Nevertheless, in the group of tobacco cigarette smokers, all the studied parameters had lower values as compared to the EC group.[151]

The latest study by Cichońska et al. indicates that EC smokers exhibit a salivary redox imbalance to the same extent as traditional cigarette smokers, expressed in a lower TAC value in the saliva of EC smokers as compared to non-smokers.[152] Although the results indicated only a slight effect on the salivary UA concentration in traditional cigarette smokers as compared to non-smokers, the UA concentration in EC users was higher than in traditional cigarette smokers and non-smokers. Disturbances in the salivary antioxidant potential may be closely related to an increased incidence of oral cancer in EC smokers, since oxygen free radicals can induce DNA damage, which can lead to neoplastic transformation.[152]

A study by Mokeem et al. suggested less harmful effects of smoking ECs, with the whole saliva concentrations of the pro-inflammatory cytokines IL-1β and IL-6 being significantly higher in traditional smokers than in EC users and non-smokers.[142] Interestingly, the interleukin levels in EC smokers reached similar values to those of non-smokers.[142] On the other hand, Ye et al. found elevated levels of the inflammatory marker PGE2 in traditional smokers as compared to EC users and the non-smoking group.[153] In contrast, Singh et al.[154] and Faridoun et al.[155] showed a significant increase in IL-1β in EC users. In addition, a higher concentration of transforming growth factor beta (TGF-β) was found in EC smokers.[155] In cancers, TGF-β expression significantly increases with the increasing tumor grade, suggesting a close relationship between this cytokine and malignant tumor changes. On the other hand, TGF-β is a cytokine released in inflammation and its level is increased in vitro by exposure to tobacco smoke. Notably, tumor development may be promoted by the altered TGF-β signaling pathway. Some previous studies reported increased levels of TGF-β in growing tumors and highlighted the prognostic properties of this cytokine.[156]

In addition to ECs, there are also heat-not-burn products on the market, which are attracting interest, especially from young smokers. According to the manufacturers, heating rather than burning tobacco is supposed to ensure less bodily exposure (including the mouth) to the harmful components of CS. Unfortunately, only one study evaluated salivary biomarkers in the smokers of heat-not-burn products. Mori et al. showed that using heat-not-burn products leads to a reduction in the secretion of unstimulated saliva.[157] They also found reduced concentrations of anti-inflammatory lactoferrin and lysozymes in smokers' saliva. These results suggest an adverse effect of heating tobacco on oral immune defense.[157]

Despite studies reporting decreased concentrations of serum antioxidants in EC users (↓ UA, ↓ carotenoids (including lutein), and ↓ α- and β-carotene) or increased levels of lipid peroxidation biomarkers in blood (↑ MDA), there is no similar data available in the context of saliva.[158] Therefore, it is difficult to determine whether traditional smoking has different effects on the oral cavity than ECs or heat-not-burn products, making further research in this area necessary (Tables S2, S4–S7, available on request from the corresponding author).

Limitations and the next steps

To our knowledge, this study is the first systematic literature review comparing the effects of traditional cigarettes, ECs and heat-not-burn products on selected saliva biomarkers. Unfortunately, the study protocol was not registered, and due to the high heterogeneity of the studies, we could not use advanced statistical methods to analyze the data, which would have increased the quality of the conclusions drawn. The inclusion and exclusion criteria were not precisely specified in all studies, and the sample size was not set a priori. Also, standardized saliva sample collection and processing procedures were not used in the analyzed studies. In addition, a variety of biochemical methods were used to assess the salivary analyte concentrations, making an objective data comparison difficult. It should also be noted that individual quantitative/qualitative changes in the saliva of smokers, in addition to the effect of tobacco smoke, may be due to other factors, such as concurrent alcohol dependence, concurrent periodontal disease, the use of prosthetic restorations, and poor oral hygiene, which may contribute to the development of the pathologies discussed. Similarly, the innate/hereditary predispositions of smokers may influence the occurrence of pathologies in saliva in response to the aforementioned stimuli. Therefore, future experiments should include randomized clinical trials on larger populations of smokers. With regard to oral and general health, long-term observations are essential. To date, the diagnostic usefulness of saliva for assessing exposure to CS has only been assessed for selected biomarkers, which also indicates the need for further research.

Conclusions

Smoking has been a substantial public health problem for years, and numerous studies have demonstrated that smoking tobacco causes damage to almost every organ of the body, including significant impairment of the functions of the oral cavity. The exposure of the oral cavity to tobacco smoke may result in reduced saliva secretion and

pH, as well as decreased immune and antioxidant salivary defense. Smoking impairs the antimicrobial properties of saliva, and increases susceptibility to caries and oxidative damage caused by the overproduction of oxygen free radicals, which is reflected in the quantitative and qualitative composition of saliva.

Some salivary biomarkers can be used to assess exposure to tobacco, including SFR, salivary pH, microbiome, and cytokines. In addition, salivary biomarkers can help assess the development of caries, the progression of periodontal disease, oral candidiasis, and oral cancer. However, the standardization of saliva sampling and processing procedures is required, as well as the development of the reference values for all salivary biomarkers.

E-cigarettes, supposedly less harmful than tobacco ones, were intended to replace the latter, and their popularity is still growing. Unfortunately, since they have only been available for a relatively short period, most of their short- and long-term effects are still unknown. Several publications report that, similar to traditional cigarettes, ECs may reduce the salivary pH or increase the levels of pro-inflammatory markers in saliva. This paper is the first systematic literature review comparing the effects of traditional cigarettes, ECs and heat-not-burn products on selected salivary biomarkers. However, there is a lack of convincing evidence to compare the toxic influence of traditional and electronic cigarettes on salivary homeostasis, with only individual salivary biomarkers in traditional and EC smokers assessed so far. Further research is necessary to fill the knowledge gap on the effects of ECs on the oral cavity. Future experiments should include randomized clinical trials on larger populations of smokers. With regard to oral health, long-term observations are essential. Furthermore, it is necessary to inform the public about the potential adverse effects of smoking in order to raise awareness of possible oral health consequences.

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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Oral lesions in adult- and juvenile-onset systemic lupus erythematosus patients: A case series report

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Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease with various oral manifestations, including ulceration, white keratotic plaques, oral discoid lupus erythematosus, oral lichen planus (OLP)-like lesions, non-specific erythema, purpura, petechiae, and cheilitis, which resemble lesions of other systemic diseases. Recognizing the oral manifestation of SLE is essential for comprehensive patient management. This study reports 4 cases of SLE with various oral lesions, underlying conditions and diagnostic methods.

In September 2019, 2 adult SLE patients and 2 juvenile SLE patients were consulted at the Oral Medicine Clinic. The assessment of systemic diseases was conducted by the Internal Medicine and Pediatrics resident, whereas the Oral Medicine resident performed the intraoral examinations. The medical history, clinical findings and laboratory results were analyzed to establish the diagnosis.

The first patient was a 38-year-old female presenting with multiple white keratotic plaques throughout the mucosa, an OLP-like lesion on the right buccal mucosa, petechiae on the hard palate, and petechiae and purpura on the upper and lower extremities. The second case was a 24-year-old female with a malar rash and multiple ulcerations on the vermilion zone, an OLP-like lesion on the left buccal mucosa, and a palatal ulcer. The third and fourth cases were 16-year-old females with a prominent butterfly rash. The patients presented with acute pseudomembranous candidiasis, an aphthous-like ulcer and keratotic plaques. They received antimicrobial therapy for the intraoral lesions and showed promising results.

The oral lesions in adult- and juvenile-onset SLE patients varied depending on the disease severity and treatment received.

Keywords: systemic lupus erythematosus, oral lesions, adult SLE, juvenile SLE, oral ulcer

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Introduction

Lupus erythematosus (LE), commonly referred to as systemic lupus erythematosus (SLE), is an autoimmune disease that can affect multiple organs and present with various clinical manifestations.[1,2] The incidence of SLE has increased in recent years, with reported rates of 2–8 cases per 100,000 individuals in Europe, South America and North America, 51 cases per 100,000 individuals in the USA,[1] and 30–50 cases per 100,000 individuals in Asia, where the incidence varied from 0.9/100,000 to 3.1% per year. The epidemiological data on SLE differs between Asian countries and is difficult to generalize. However, there are similarities in the clinical presentation of the disease.[3] In 2010, 291 SLE patients were registered at the Rheumatology Clinic of Dr. Hasan Sadikin Central General Hospital (Bandung, Indonesia), accounting for 10.5% of all patients registered at the Rheumatology Clinic.[1]

In 1997, the American College of Rheumatology (ACR) issued a set of diagnostic criteria for SLE, which were revised in 2012 by the Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) to be more sensitive but less specific.[4] These criteria are used to diagnose SLE in children and adults. Several studies reported that oral lesions in SLE varied from 9% to 45% and from 3% to 20% in localized cutaneous disease.[5–7] In 2019, the European League Against Rheumatism (EULAR) and the ACR approved new criteria for SLE, which require a positive antinuclear antibody (ANA) test. The new criteria have 96.1% sensitivity and 93.4% specificity, compared with 82.8% sensitivity and 93.4% specificity of the ACR 1997 criteria and 96.7% sensitivity and 83.7% specificity of the SLICC 2012 criteria.[8]

Many terms have been used to describe the LE oral lesion, including oral discoid lesion, chronic plaque, lupus cheilitis, acute ulcer, oral ulcer, red ulcer, ulcerative plaques, pebbly red area, honeycomb lesion, keratotic lesion, white keratotic plaques, purpuric lesion, and diffuse palatal petechial erythema.[9–13] A study in the Hungarian population reported that lupus nephritis, hematological disorders, photosensitivity, butterfly rash, and mucosal ulceration are more common in children than in adults, whereas neurological symptoms and polyarthritis occur more frequently in adults.[14] The present article describes 4 clinical cases of various oral lesions identified in patients with SLE, along with their diagnosis and management.

Material and methods

This article is a case series study with a prospective design conducted in a single center using consecutive sampling. The patients were admitted to Dr. Hasan Sadikin Central General Hospital (Bandung, Indonesia), a government-run academic and community hospital. An Internal Medicine and Pediatrics resident, supervised by their consultant, performed the examinations of systemic conditions and laboratory assessments needed to support the diagnosis. At the same time, an Oral Medicine resident conducted intraoral examinations under the supervision of their consultant. The final diagnosis was based on patient complaints, clinical (extraoral and intraoral) observations and laboratory findings. The clinicians provided medication for systemic diseases, whereas intraoral lesions were treated by oral medicine specialists. Patient improvement was followed up for 3–4 weeks and any changes in medication type or dosage were reported. The results of the examinations, diagnosis, treatment, and oral lesion progress were documented in the patient's medical records. The patients provided consent to document the intraoral lesions for further evaluation.

Results

The case series involved 4 female patients admitted to Dr. Hasan Sadikin Central General Hospital between September 2019 and December 2019. All patients were of reproductive age. Table 1 describes the clinical characteristics of each patient, with a butterfly (malar) rash and extraoral pale conjunctiva being the most common.

Table 1. Clinical features present in patients with systemic lupus erythematosus (SLE)

Variable	Case 1	Case 2	Case 3	Case 4
Age [years]	38	24	16	16
Gender	female	female	female	female
Extraoral findings	• pale conjunctiva • petechiae and purpura on the upper and lower extremities	• butterfly rash • pale conjunctiva • swelling and ulceration of the lips	• butterfly rash • pale conjunctiva • alopecia • exfoliation of the vermilion zone	• butterfly rash
Intraoral findings	• keratotic plaques • non-specific petechiae • unpainful ulceration surrounded by whitish striae (OLP-like lesion)	• multiple ulcerations • acute pseudomembranous candidiasis • central palatal erythema	• acute pseudomembranous candidiasis • unpainful ulceration surrounded by whitish striae (OLP-like lesion)	• multiple ulcerations • keratotic plaques

OLP – oral lichen planus.

Intraoral manifestations ranged from aphthous-like ulcers, keratotic plaques and oral lichen planus (OLP)-like lesions to central palatal erythema and acute pseudomembranous candidiasis. All patients were diagnosed with SLE according to the SLICC criteria, which are summarized in Table 2.

The first patient presented with secondary Evans syndrome, and the diagnosis of SLE was confirmed following intraoral examination and ANA testing, which indicates that intraoral findings may play a role in establishing a definitive diagnosis. Extraoral findings included petechiae and purpura on the upper and lower extremities, and pale conjunctiva (Fig. 1A,B). Intraoral lesions included keratotic plaques on the upper and lower labial mucosa and the left and right lateral border of the tongue that could not be scraped (Fig. 1C–F), unpainful lesions on the right buccal mucosa surrounded by whitish reticular plaque (OLP-like lesions) (Fig. 1G), whereas the left buccal mucosa showed no striae (Fig. 1H). Multiple petechiae were also observed on the hard palate. The patient received the treatment listed in Table 3, was discharged 5 days later, and continued as an outpatient at a hospital closer to her hometown.

The second patient was diagnosed with SLE in April 2009 at another hospital, and was undergoing SLE treatment.

Table 2. Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) criteria used for the patients' diagnosis

SLICC criteria		Case 1	Case 2	Case 3	Case 4
Clinical criteria	acute cutaneous lupus	yes	yes	yes	yes
	chronic cutaneous lupus	no	no	no	no
	oral or nasal ulcers	yes	yes	yes	yes
	non-scarring alopecia	no	no	yes	no
	arthritis	no	no	yes	yes
	serositis	no	no	no	no
	renal	no	no	no	no
	neurologic	no	no	no	no
	hemolytic anemia	yes	yes	yes	yes
	leukopenia	yes	yes	yes	yes
	thrombocytopenia	yes	yes	yes	yes
Immunologic criteria	ANA	reactive	reactive	reactive	reactive
	anti-DNA	not done	not done	not done	not reactive
	anti-Sm	not done	not done	not done	not done
	antiphospholipid antibody	not done	not done	not done	not done
	low complement (C3, C4, CH50)	not done	not done	not done	not done
	direct Coombs test	not done	not done	not done	not done

ANA – antinuclear antibody; anti-Sm – anti-Smith.

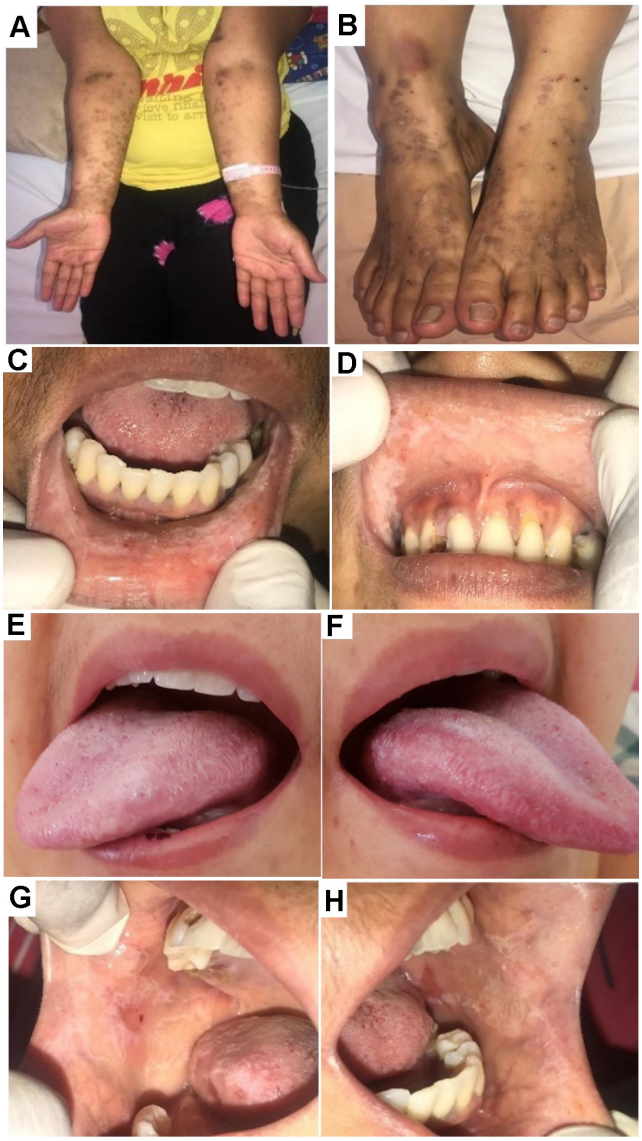

Fig. 1. A,B. Petechiae and purpura on the upper and lower extremities; C,D. Non-scrapable whitish plaques on the upper and lower labial mucosa; E,F. Non-scrapable whitish plaques on the left and right lateral border of the tongue; G. Unpainful ulceration on the right buccal mucosa surounded by whitish striae (oral lichen planus (OLP)-like lesion); H. Unpainful ulcer with whitish keratotic plaques

She complained of swelling and ulceration of the lips. A few months earlier, she had similar symptoms after taking an antibiotic, which was confirmed to be an allergic reaction. The patient stated that the current swelling was not related to any medication. Extraoral findings revealed pale conjunctiva, a butterfly rash (Fig. 2A) and swelling of the lips associated with multiple minor ulcerations on the lower labial mucosa (Fig. 2B). Intraoral examination showed multiple ulcerations on the upper and labial mucosa as well as the left and right buccal mucosa. Additionally, acute pseudomembranous candidiasis (Fig. 2C) and a central erythematous lesion on the hard palate intermixed with whitish pseudomembranous plaques (Fig. 2D) were observed. The differential diagnosis consisted of herpes-associated erythema

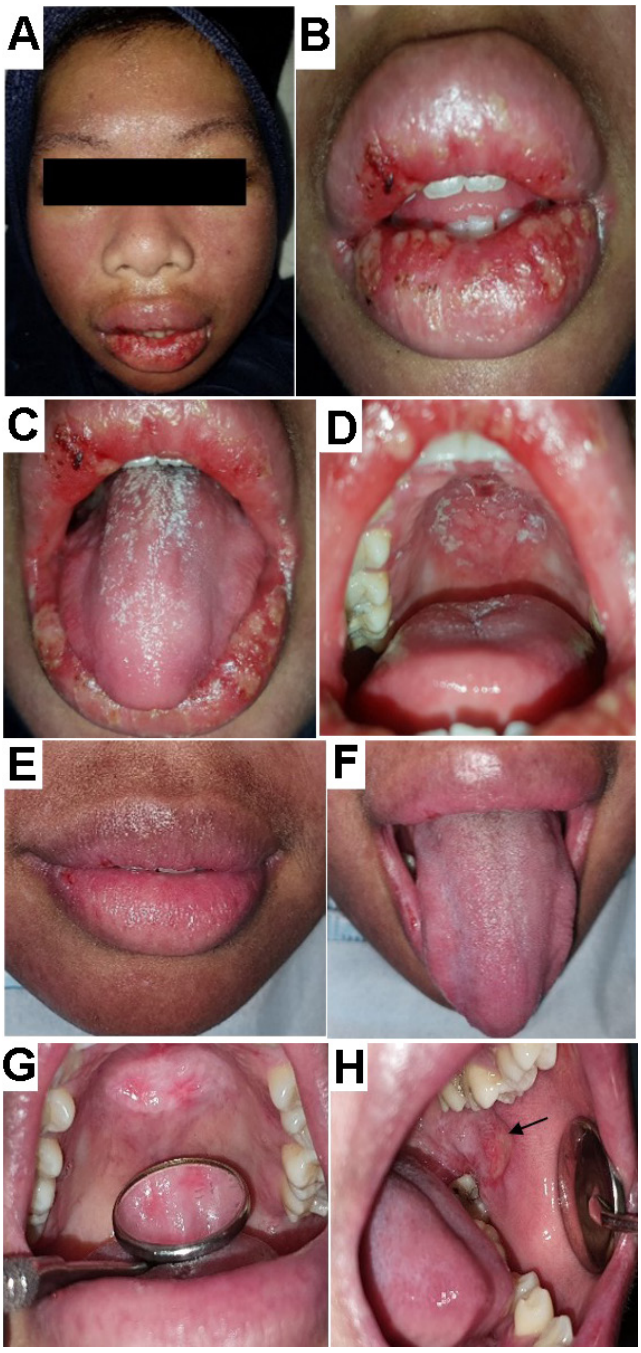

Fig. 2. A. Butterfly rash; B. Swelling on the lower labial mucosa associated with multiple ulcerations; C,D. Acute pseudomembranous candidiasis on the dorsum of the tongue and palate; E. Lip swelling subsided; F. Whitish plaques disappeared; G. Central erythematous lesion on the hard palate; H. Whitish radiant striae with central ulceration resembling oral lichen planus on the left buccal mucosa

multiforme and drug-induced erythema multiforme. Herpes simplex virus-1 (HSV-1) and immunoglobulin E (IgE) serology were performed to rule out any possibility of hypersensitivity reaction and HSV involvement. The results for IgE were within the normal range and serology for HSV-1 was non-reactive. Therefore, intraoral HSV infection was excluded. Table 3 summarizes the patient's medications.

One week later, the lip swelling, ulceration and oral candidiasis (Fig. 2E,F) subsided. However, an unpainful central erythematous lesion on the hard palate (Fig. 2G) was revealed. Additionally, whitish radiant striae with central ulceration resembling OLP were observed on the left buccal mucosa (Fig. 2H). The patient was instructed to continue using the antimicrobial gel for the lesion on the palate and left buccal mucosa and petroleum jelly to reduce lip dryness. Selective grinding was performed on teeth 27 and 36 to minimize traumatic contact with the ulcer on the left buccal mucosa.

The third patient reported difficulty eating due to multiple ulcerations in the mouth. She had been treated for pulmonary tuberculosis for the past 2 months. However, a thoracic X-ray examination revealed that the pulmonary tuberculosis was not active and there was no cardiomegaly. Upon examination, we observed a prominent butterfly rash (Fig. 3A), pale conjunctiva, alopecia, dryness, and exfoliation of the vermilion. Intraoral findings revealed multiple scrapable white patches, leaving an erythematous base on the lower labial mucosa, hard palate and left buccal mucosa (Fig. 3B–D). A thick coating was present on the dorsum of the tongue (Fig. 3E). Laboratory findings showed hemolytic anemia, elevated blood glucose, serum glutamic-oxaloacetic transaminase (SGOT), urea, and creatinine levels, and a reactive ANA test. Table 3 lists the patient's medications.

Two weeks after treatment, the patient's condition improved. The coated tongue showed marked improvement (Fig. 3F), the dryness of the vermilion decreased (Fig. 3G), and the oral candidiasis was reduced and almost completely resolved (Fig. 3H–J). The patient was discharged and scheduled for regular control at the Pediatric Department, having followed the instructions for maintaining oral hygiene.

The fourth patient reported experiencing ulcerations in the mouth, pain while swallowing and pain inside the ear

Table 3. Treatment of patients with systemic lupus erythematosus (SLE)

Case 1	Case 2	Case 3	Case 4
• systemic corticosteroid • calcium carbonate • pulse dose intravenous dexamethasone • omeprazole • oral topical corticosteroid • antimicrobial mouthwash	• antifungal oral suspension • antimicrobial oral gel • petroleum jelly • calcium carbonate • vitamin D3 • selective grinding (teeth 27 and 36)	• intravenous corticosteroid • calcium carbonate • antifungal oral suspension • oral hygiene instructions	• intravenous corticosteroid • antibiotics • calcium carbonate • vitamin D3 • skin topical corticosteroid • SPF 45 sunblock • antimicrobial mouthwash

SPF – sun protection factor.

for 2 days before admission. The patient has been receiving treatment for SLE with skin and musculoskeletal involvement since March 2019. On examination, a butterfly rash was observed on her face (Fig. 4A). Intraorally, minor ulcerations surrounded by an erythematous area and a regular border were found on the lower labial mucosa opposite tooth 32 (Fig. 4B). Multiple faint, non-scrapable whitish plaques were observed at the anterior part of the left and posterior part of the right buccal mucosa (opposite teeth 44 and 46), and on the right and left lateral borders of the tongue (Fig. 4C–E). The patient's treatment is described in Table 3. Five days later, the ulceration on the lower labial mucosa disappeared, but the white keratotic plaques on the tongue and the faint whitish plaques on the right buccal mucosa persisted.

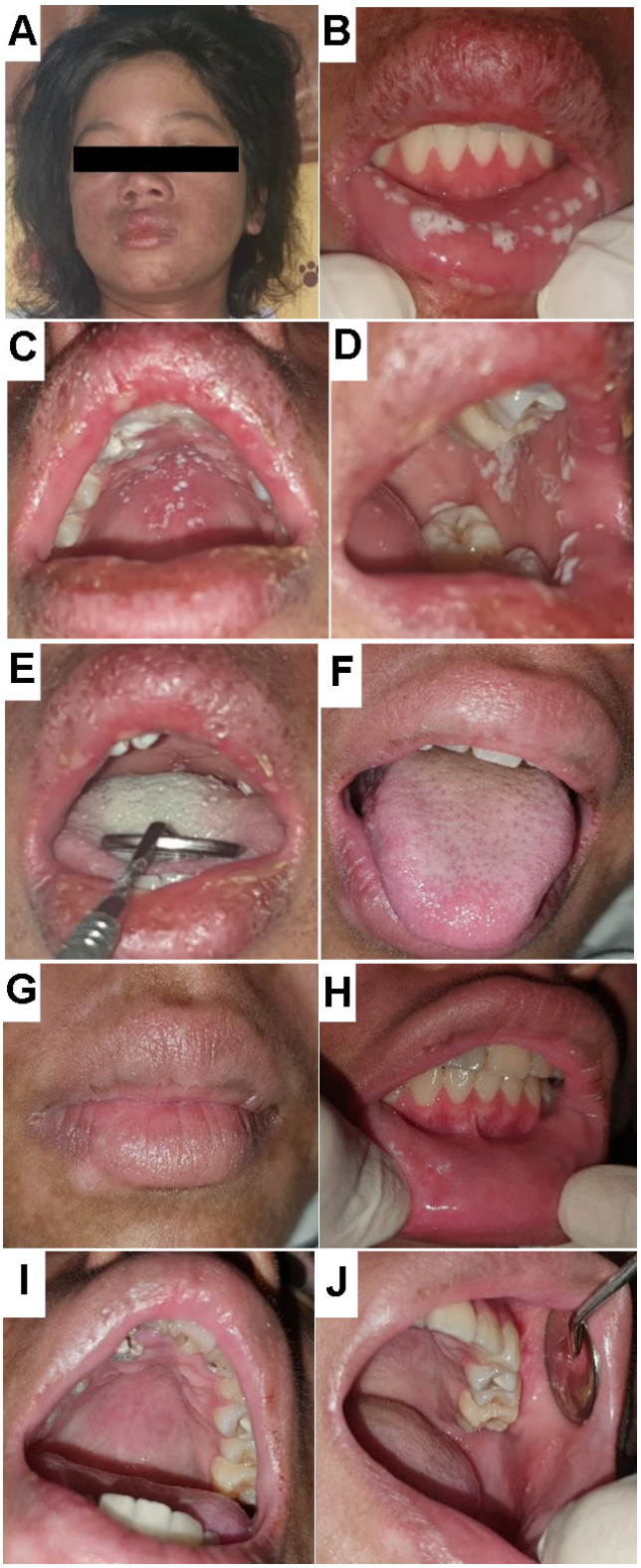

Fig. 3. A. Butterfly rash; B. Multiple scrapable white patches that leave an erythematous base on the lower labial mucosa; C,D. Multiple scrapable white patches that leave an erythematous base on the hard palate and left buccal mucosa; E. Thickly coated tongue; F. Coated tongue improved; G. Dryness of the vermilion decreased; H. Whitish plaques decreased; I,J. Whitish plaques almost completely resolved

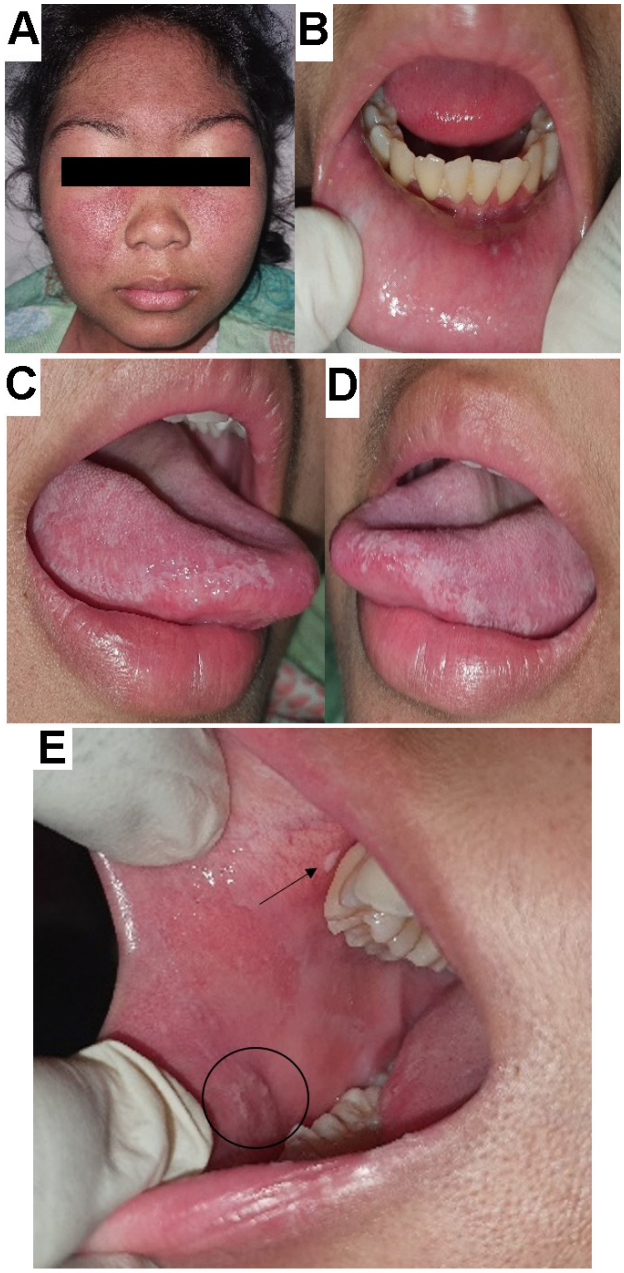

Fig. 4. A. Butterfly rash; B. Minor ulcer on the lower labial mucosa; C,D. Multiple faint non-scrapable whitish plaques on the right and left lateral borders of the tongue; E. Multiple faint non-scrapable whitish plaques (marked with a circle) and a minor ulcer (marked with an arrow) on the right buccal mucosa

Discussion

Oral ulceration can be an indicator of various systemic diseases, such as LP, LE, benign mucous membrane pemphigoid, pemphigus vulgaris, Crohn's disease, and Behçet's syndrome. The ulcerations may present in various ways but maintain their characteristic features. In LE, oral lesions may present as oral discoid lesions, erythema, irregularly shaped ulcers, honeycomb plaques, raised keratotic plaques, purpura, petechiae, and cheilitis.[15] These lesions may also be accompanied by other organ involvement or present as a solitary lesion. Therefore, it is crucial for clinicians to recognize the characteristic presentation of the disease to achieve an accurate diagnosis.

The first classifications of mucocutaneous SLE in the 1970s were divided into lupus-specific skin lesions (LE-specific) and non-specific skin lesions (LE-non-specific) and are used in both adult- (adult SLE) and juvenile-onset SLE (JSLE).[16] The majority of the manifestations appear similarly in both groups. A study reported that the LE-specific butterfly rash and the generalized lupus rash were more frequent in JSLE than in adult SLE.[17] In contrast, adult SLE patients were more likely than JSLE patients to present with subacute cutaneous lesions, discoid rash, generalized discoid LE (DLE), and LE panniculitis (profundus). In LE-non-specific cases, cutaneous vasculitis, oral and nasal ulcers, and bullous SLE were more common in JSLE patients, whereas photosensitivity, non-scaring alopecia, livedo reticularis, and Raynaud's phenomenon were more frequent in adult SLE.[17]

The most common LE-specific lesion in JSLE and adult SLE is the butterfly rash. It presents as a symmetrical erythematous and edematous non-pruritic rash over the nasal bridge, typically sparing the nasolabial folds. This rash represents acute cutaneous lupus erythematosus (ACLE).[18–20] In the present report, the butterfly rash was present in 3 out of 4 cases, with 2 patients reporting a recent diagnosis of SLE within the previous 2 weeks or 6 months.

Diagnosing SLE in the first patient was more challenging due to the absence of a butterfly rash. The patient presented with rashes on the upper and lower extremities, which are not specific to cutaneous LE. However, the presence of oral ulcers and laboratory findings of hemolytic anemia, leukopenia, thrombocytopenia, and a reactive ANA test helped establish the correct diagnosis. As stated in the literature, secondary Evans syndrome is linked to suspected underlying autoimmune diseases such as SLE, which causes hemolytic anemia, leukopenia and thrombocytopenia.[21]

In cases of adult SLE, oral ulcers may resemble reticular and erosive OLP, which typically present as a white lacy patch or Wickham's striae and erythematous, ulcerated or erosive mucosa, frequently on the bilateral buccal mucosa and rarely on the palate. They may also resemble oral lichenoid reactions, which are usually associated with an adjacent metallic dental restoration. A biopsy of the lesion is required when the clinical presentation alone cannot establish a definitive diagnosis.[22] However, in our study, a biopsy could not be performed in the first patient due to hemolytic anemia and thrombocytopenia, while the second patient refused to undergo the procedure. Moreover, the OLP-like lesions observed in our SLE patients were painless, unlike the erosive OLP, which may cause symptoms and interfere with patient activities.

Lip swelling may resemble angioedema, but it does not need to be accompanied by multiple ulcerations as observed in the second case. Erythema multiforme associated with HSV or induced by some drugs was also considered as a differential diagnosis due to the patient's recent history of similar lip swelling associated with antibiotic treatment. However, we excluded the possibility of a hypersensitivity reaction due to the normal range of the serology test and the absence of causative antibiotic or drug consumption. Fortunately, the swelling subsided within a few days and the ulcerations, which may have been non-specific, healed.

The third patient did not have any SLE oral lesions, except for oral candidiasis. Multiple risk factors have been identified for oral candidiasis in SLE patients. A study recommends examining for oral candidiasis in those with active disease, proteinuria, high white blood cell count, and those taking prednisone, immunosuppressive agents or antibiotics.[23] In our study, the third patient had proteinuria, was taking prolonged methylprednisolone and antibiotics for SLE, and had pulmonary tuberculosis. Therefore, he was at increased risk of opportunistic infection, such as oral or oropharyngeal candidiasis.

The fourth case demonstrated LE-non-specific aphthous oral ulcers. The white keratotic plaque observed at the lateral border of the tongue may indicate initial verrucous LE. This white plaque-like lesion may resemble homogeneous leukoplakia or reticular OLP.[24] In 2005, the World Health Organization (WHO) defined leukoplakia as "a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer".[25] In contrast, there were no changes in the tissue's physical consistency, which is typically identified in leukoplakia.

In the present report, the diagnosis of SLE adhered to diagnostic criteria established by the SLICC. The disease activity was measured using the MEX-SLEDAI (Mexican Version of Systemic Lupus Erythematosus Disease Activity Index). The third and fourth cases had index scores of 17 and 2, respectively. A score greater than 5 indicates an active or flare condition, a score of 2–5 suggests the possibility of a flare, and a score of less than 2 represents an inactive condition or remission.[26] Regrettably, the disease activity was not evaluated in the first and second cases.

The management of SLE involves the use of corticosteroids and an antimicrobial mouthwash to suppress the autoantibody response. Chlorhexidine digluconate (0.12%) was used as an antimicrobial in this case series due to its antiplaque properties, bactericidal action to prevent secondary infection of oral ulcers and promising antifungal benefits.[27] The importance of a multidisciplinary approach and referral should be emphasized in the management of multi-organ SLE as it results in a shorter recovery time and improved patient quality of life.[28,29]

The article described various oral lesions in adults and juveniles with SLE and their management. However, only 4 SLE cases were reported with limited follow-up on the patients' systemic conditions. Additional research should be conducted with a larger sample size and a longer follow-up period.

Conclusions

In conclusion, oral manifestations in adult- and juvenile-onset SLE have a diverse presentation and may resemble lesions in other diseases. Additionally, they may occur as a manifestation of treatment received for the underlying systemic disease. The lesions presented in this report were mostly ulcerations, including aphthous-like ulcers, OLP-like lesions, palatal ulcers, erythema, and white keratotic honeycomb plaques, which may also indicate the underlying disease. Oral candidiasis may appear as a side effect of systemic condition treatment. A thorough evaluation of oral and systemic conditions is necessary for the accurate diagnosis and comprehensive management of patients with adult- and juvenile-onset SLE.

Ethics approval and consent to participate

The authors confirm that they have obtained all necessary patient consent forms. The patients were informed that their names and initials would not be published, and efforts would be made to conceal their identity.

Data availability

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

Consent for publication

The patients have given their consent for the publication of medical images presented in the article.

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Research letter

Initial assessment of the psycho-emotional state of patients with temporomandibular disorders: A pilot study

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

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Conflict of interest
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Abstract

Background. Temporomandibular disorders (TMD) are a group of conditions that affect the function of the masticatory muscles, temporomandibular joints and surrounding structures.

Objectives. The objective of the preliminary investigation was to develop an initial questionnaire for emotional assessment, pre-designed for TMD patients, and provide guidance for further management through referral to psychological and/or psychiatric counseling. Additionally, we aimed to compare the results of tests carried out in TMD patients with those of healthy subjects.

Material and methods. The study involved 260 patients who reported for dental treatment. The TMD study group (Group 1, $n = 130$) consisted of patients diagnosed with TMD, and the control group (Group 2, $n = 130$) had TMD diagnostically excluded. The questionnaire included 30 questions about the emotional state of the patients in the past 4 weeks.

Results. The mean scores were 37.715 points for Group 1 (median (Me) = 35.5, standard deviation (SD) = 12.58 and 24.938 points for Group 2 ($Me = 24$, $SD = 7.95$) ($p < 0.001$).

Conclusions. The research suggests that the developed questionnaire is useful for an initial assessment of the psycho-emotional state of TMD patients. Furthermore, the results emphasize a greater need for psychological counseling in TMD patients compared to their healthy counterparts.

Keywords: anxiety, stress, questionnaire, temporomandibular disorders, TMD

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Introduction

Temporomandibular disorders (TMD) are a group of clinical conditions that affect the function of the masticatory muscles, temporomandibular joints and the surrounding structures. They are a significant cause of orofacial pain, along with dental pain. In TMD, pain is often caused by overloading the masticatory muscles rather than by primary joint changes. Temporomandibular disorders have a multifactorial and complex etiopathogenesis.[1–3] Psycho-emotional disorders (excessive nervous excitability, anxiety and depression) are a critical etiological factor of this condition, as confirmed by numerous studies.[4–11] However, several other factors may also contribute to TMD development.

An increase in stress levels (psycho-emotional tension) is a crucial factor affecting the state of masticatory function. Structures such as the hypothalamus, the reticular formation and the limbic system have a decisive influence on the patient's emotional state.[1,2,12–15] The activity of the limbic system, which governs emotions, and the additional connections of the gamma loops to the masticatory muscles, determine that increased emotional tension results in a significant increase in the contractile activity of the masticatory muscles.[16]

Temporomandibular disorders and functional disorders of the masticatory muscles are the most common complaints of patients seeking treatment in the dental office. The primary symptoms of TMD are pain and dysfunction, with myalgia being most commonly caused by increased muscle use, which is related to arterial vasoconstriction and accumulation of metabolic waste products in the muscles. Activities such as daytime teeth clenching, gum chewing, and biting lips, fingernails or cheeks cause significant strain on the masticatory muscles and temporomandibular joints.[1,15] Karacay and Sahbaz evaluated the relationship between TMD type and the occurrence of probable sleep bruxism and awake bruxism, demonstrating an association with TMD-related pain and intra-articular joint disorders.[16]

The relationship between psychological factors and clinical pain is well established. Numerous cross-sectional studies show that individuals with chronic pain exhibit higher levels of psychological distress, environmental stress and somatic symptoms than those without pain.[7–9,17,18] Additionally, chronic TMD cases differ from controls in terms of personality traits, such as neuroticism.[6–9,17,18] Diagnostic procedures use the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) questionnaire axis II (biobehavioral questionnaires), which predominantly focuses on pain assessment, with the patient's emotional state (point 20) evaluated to a very limited extent.[2] Despite its widespread use, the questionnaire does not provide a reliable multidirectional assessment of psycho-emotional status. It may provide broad insight into potential psychological disturbances in chronic TMD, such as mood disorders, anxiety disorders and psychosocial disability. However, it does not offer guidance on further psychological, psychotherapeutic and/or medical support. Wieckiewicz et al. emphasized the importance of psycho-emotional factors, such as stress, fatigue, anxiety, depression, sleep disorders, and the fast pace of life, which have a significant negative impact on the human psyche and the progression of TMD.[8]

The aims of the preliminary investigation were:

1) to develop an initial questionnaire for the emotional assessment of patients with TMD and provide guidance for further management, including specialized psychological and/or psychiatric consultation;
2) to compare the results of tests carried out on TMD patients with those of healthy subjects without masticatory dysfunction symptoms.

The primary goal of the project was to create a universal questionnaire for the initial evaluation of the psycho-emotional state of TMD patients that would be available to all dentists as an alternative to numerous assessment scales, such as the Beck Anxiety Inventory (BAI), the Perceived Stress Scale (PSS), the Patient Health Questionnaire-9 (PHQ-9), the Symptom Checklist 90-Revised (SCL-90-R), the State-Trait Anxiety Inventory (STAI), and the Lazarus–Folkman Ways of Coping Questionnaire.[4,19] The questionnaire is only intended as a preliminary assessment of the psycho-emotional state to guide the dentist's further management of psychotherapeutic support for TMD patients. It refers to broad emotions such as lowered mood, anxiety, introversion, current mental well-being, sudden panic attacks, and self-dissatisfaction.

Material and methods

The study involved 260 patients who reported for dental treatment at the Prosthodontics Clinic of the Institute of Dentistry at Jagiellonian University in Krakow, Poland, and the Prosthodontic Cilnic at Medical University of Warsaw, Poland, between October 2021 and October 2023. Permission to conduct the study using our self-developed questionnaire was granted by the Bioethics Committee of Jagiellonian University (consent No. 1072.6120.312.2021 – 15.12.2021; clinical trial No. 1072.6120.312.2021 – 15.12.2021; ID NCT06041633). The population consisted of both men and women, with a female predominance, aged between 20 and 43 years. The study group (Group 1) consisted of 130 patients diagnosed with TMD, whereas the control group (Group 2) consisted of 130 patients who were excluded from having TMD (confirmed or excluded using the RDC/TMD questionnaire). The prosthetic treatment of patients in Group 2 was necessary to replace single missing teeth.

The inclusion criteria for the study were:

1) good general health, with no craniofacial trauma in the last 5 years and no severe mental health conditions;
2) the presence of TMD symptoms (pain in the masticatory muscles, limitation of the mouth opening range, and clicking or popping in the temporomandibular joints); these symptoms were not present in Group 2;
3) the patient's consent to participate in the research project.

The exclusion criteria for the study were:

1) willingness to withdraw from the study;
2) the presence of general medical conditions that made it impossible to continue participation in the study.

The questionnaire included 30 questions related to the respondent's emotional state over the past 4 weeks, such as lowered mood, emotional irritability, feelings of sadness, lack of desire for daily activities, lack of concentration, a desire for self-isolation, critical thoughts toward oneself, nervousness, or anxiety. Responses to each question included: no, several times in the last 4 weeks, several times per week, several times a day, and constantly/continuously. The results of the questionnaire were divided into sections A, B, C, and D, with scores ranging from 0 (most favorable response) to 4 (most unfavorable response) for each question.

During the initial stage of questionnaire development, it was evaluated for the appropriateness of the questions (special questionnaire). This evaluation was carried out by 4 dentists and 4 psychologists who were invited to cooperate, all of whom provided positive feedback. The survey was linguistically adapted by first being translated from Polish to English by a sworn translator, and then translated back into Polish by a person of British origin who is well-versed in Polish and works in Poland. Upon comparison of the 2 versions, differences were found in the wording of memory disorders.

The results of the questionnaire were compiled as follows:

– section A (0–30 points): good psycho-emotional state, possible individual difficulties with negative emotions;
– section B (31–60 points): mild psycho-emotional difficulties, symptoms indicating the experience of a small degree of emotional difficulties on a daily basis that do not cause discomfort to the patient but require monitoring, with a repeat examination indicated in about a few months;
– section C (61–90 points): moderate psycho-emotional difficulties that may cause discomfort to the patient if they increase in severity or persist (more than 3 months). Specialized help is recommended through psychoeducation and psychological and psychotherapeutic counseling;
– section D (91–120 points): exacerbated psycho-emotional difficulties that are frequent or continuous in nature. Psychiatric consultation and psychotherapeutic support are indicated.

Statistical analysis

Statistical analysis employed the IBM SPSS Statistics for Windows software, v. 29.0 (IBM Corp., Armonk, USA). The normality of data distribution was assessed using the Shapiro–Wilk test, and descriptive statistics, including mean (*M*) and standard deviation (*SD*), were calculated. Since the data was not normally distributed, differences between clinical cases and the control group were compared using the non-parametric Mann–Whitney U test. The χ^2 test verified any relationship between the categories (with points' partitions) between the 2 groups. The differences were considered significant for $p < 0.05$.[20]

A normality test determines whether the sample data was drawn from a normally distributed population. The Kolmogorov–Smirnov and Shapiro–Wilk tests are commonly used to test data normality. Since the data was not normally distributed, non-parametric tests were used (Mann–Whitney U test and χ^2 test).

The Mann–Whitney U test was implemented in SPSS software to test the null hypothesis that there are no statistically significant differences between the scores of 2 population groups. This function takes 2 data samples as parameters, uses the median (*Me*) as a measure of central tendency, and returns the test results with a *p*-value to indicate statistical significance. A significance level of $p < 0.05$ was used for all analyses, as it is commonly used in biomedical research.

The χ^2 test was utilized as a statistical tool to assess whether 2 categorical variables were related or independent and to determine if the observed data significantly differed from the expected data. By comparing the 2 datasets, we can draw conclusions about whether the variables have a meaningful association. Additionally, sensitivity and specificity analyses were conducted.

Sensitivity and specificity are statistical measures commonly used in diagnostic and classification tasks to evaluate the performance of a statistical test or model. They are used to distinguish between 2 groups, such as control and clinical/study groups. In the context of questionnaire data, sensitivity and specificity can help assess how well the questionnaire can identify individuals in the clinical group while minimizing false positive cases in the control group. Receiver operating characteristic (ROC) analysis was used for these calculations.

Bias

A potential source of error in a survey is the provision of false answers, which may occur due to the inclusion of personal information, such as the respondent's name, or the presence of sensitive questions, such as those related to suicidal thoughts or the use of alcohol and other psychoactive substances.

Results

The results showed that Group 1 (TMD patients) had a significantly higher percentage of responses that were graded as B (90 patients/69.2%) and C (10 patients/7.7%) compared to Group 2 (controls). In Group 1, 29 patients (22.3%) scored an A and 1 individual scored a D (0.8%).

In Group 2, 101 individuals (77.7%) scored an A, 28 patients (21.5%) scored a B, 1 patient scored a C, and there were no scores of D. These results show significantly more scores of B in Group 1 and A in Group 2. The results are collated in Tables 1–3 and Fig. 1.

The mean score obtained in Group 1 was 37.715 points (median (*Me*) = 35.5, standard deviation (*SD*) = 12.58), while Group 2 had a mean score of 24.938 points (*Me* = 24, *SD* = 7.95) ($p < 0.001$). Most respondents in Group 1 received a score of 40 (diagnosis B), while most respondents in Group 2 scored 20, which is within the range of scores for individuals without psycho-emotional disorders.

Upon analyzing the resulting diagnoses (A, B, C, and D) and the need for psychological or psychiatric support, significant differences were found in diagnoses A, B and C between Group 1 and Group 2, while the results for D did not differ. These findings suggest the importance of simultaneous diagnosis of TMD and evaluation of the patient's psycho-emotional status due to the significant contribution of this factor to the etiology of TMD. The results of the RDC/TMD diagnosis within Group 1 are presented in Table 4 and Fig. 2.

The ROC curve analysis was performed to determine the cut-off point for the questionnaire scores. On this basis, the entire model was analyzed and found to be of good quality (Table 5) (Fig. 3). Based on the Youden index (0.554) and the Gini index (0.684) as classifier evaluation metrics, a cut-off point of 29.5 was established for the questionnaire. The sensitivity and specificity values for the cut-off point were both 0.777, indicating that 78% (n = 101) of the cases were correctly classified into the study group. Therefore, those who scored 29.5 points and

Table 1. Descriptive statistics of the developed questionnaire scores for Groups 1 and 2

Variable		Group 1		Group 2	
		Statistic	*SE*	Statistic	*SE*
M		37.72	1.104	24.94	0.697
95% *CI* for *M*	lower bound	35.53	–	23.56	–
	upper bound	39.90	–	26.32	–
5% trimmed mean		36.81	–	24.54	–
Me		35.50	–	24.00	–
Variance		158.329	–	63.190	–
SD		12.583	–	7.949	–
Minimum		17	–	10	–
Maximum		92	–	68	–
Range		75	–	58	–
IQR		8	–	10	–
Skewness		1.493	0.212	1.532	0.212
Kurtosis		3.245	0.422	6.006	0.422

Group 1 – patients with temporomandibular disorders (TMD); Group 2 – control group; *M* – mean; *CI* – confidence interval; *Me* – median; *SD* – standard deviation; *IQR* – interquartile range; *SE* – standard error.

Table 3. Distribution of questionnaire sections established as identification of psycho-emotional or psychiatric support needs in Groups 1 and 2

Classification	Group 1	Group 2	Total
Section A	29 (22.3)	101 (77.7)	130 (50)
Section B	90 (69.2)	28 (21.5)	118 (45.4)
Section C	10 (7.7)	1 (0.8)	11 (4.2)
Section D	1 (0.8)	0 (0.0)	1 (0.4)
Total	130 (100.0)	130 (100.0)	260 (100.0)

Data presented as number (percentage) (*n* (%)). There are significant differences between the groups in sections A, B and C.

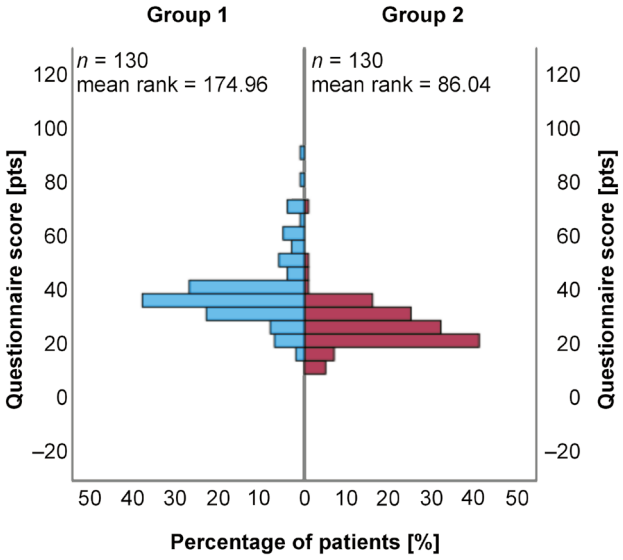

Fig. 1. Frequency of questionnaire scores in Groups 1 and 2

Group 1 – patients with temporomandibular disorders (TMD); Group 2 – control group. Independent-samples Mann–Whitney U test was used.

Table 2. Statistical analysis of mean questionnaire scores in Groups 1 and 2

Group	Patients, *n*	*M*	*Me*	Minimum	Maximum	*SD*	*p*-value
Group 1	130	37.715	35.500	17.000	92.000	12.580	<0.001*
Group 2	130	24.938	24.000	10.000	68.000	7.950	

* statistically significant (Mann–Whitney U test).

higher on the questionnaire were more likely to be classified into the study group than those who obtained lower results.

Table 4. Statistical analysis of the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) diagnosis results in Group 1

Variable	Result
Patients, *n*	130
Test statistic	5.053
df	5
Asymptotic significance (two-sided test)	0.409

df – degrees of freedom.

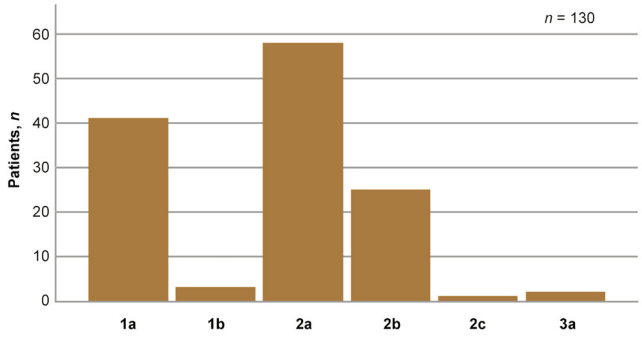

Fig. 2. Distribution of TMD forms diagnosed using the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) questionnaire in Group 1

1a – myofascial pain; 1b – myofascial pain with limited opening; 2a – disc displacement with reduction; 2b – disc displacement without reduction with limited opening; 2c – disc displacement without reduction, without limited opening; 3a – arthralgia.

Table 5. Analysis of the area under the receiver operating characteristic (ROC) curve

Area	*SE*	Asymptotic significance	Asymptotic 95% *CI*	
			lower bound	upper bound
0.842	0.025	0.000	0.793	0.891

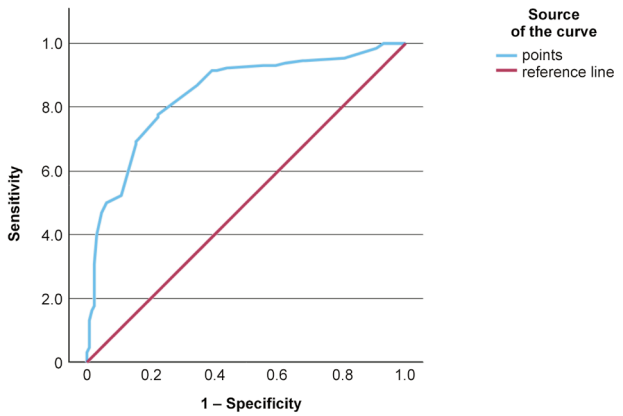

Fig. 3. Receiver operating characteristic (ROC) curve analysis for sensitivity and specificity of study groups

More individuals in Group 1 scored 29.5 points or higher compared to Group 2, and more individuals in Group 2 scored less than 29.5 points compared to Group 1. This difference was found to be statistically significant using the χ^2 test and Fisher's exact test (Table 6).

Table 6. Comparison of classification results based on statistical variables between Groups 1 and 2

Variable	χ^2 test		
	value	*df*	*p*-value
Pearson's χ^2	79.754	1	<0.001*
Continuity correction	77.554	1	<0.001*
Likelihood ratio	84.434	1	<0.001*
Fisher's exact test	–	–	<0.001*
Linear-by-linear association	79.447	1	<0.001*
Valid cases, *n*	260	–	–

* statistically significant.

Discussion

The results of the current study suggest that the developed questionnaire is useful for the initial assessment of the psycho-emotional state of TMD patients. The information obtained, in accordance with established indications for further psychological or psychiatric consultation, can be an invaluable addition to the diagnosis of TMD patients and may constitute a critical element for effective treatment of the disorder. The results are significant and suggest that patients with TMD suffer from psycho-emotional problems and require more frequent consultation and psychological support than healthy individuals.

Given the numerous reports in the literature linking the psycho-emotional disorders with TMD development,[1,2,5,6,8,12,16,18,21–25] it is important to consider the patient's condition during specialized diagnosis of this disease. In recent years, emotional state assessment has become even more important due to the significant stress and emotional strain experienced by a significant portion of the population as a result of coronavirus disease 2019 (COVID-19), including the death of loved ones and concern for their lives.[19]

In their cross-sectional analysis of Swedish national registries, Fredricson et al. demonstrated a strong association between the occurrence of mental and behavioral disorders (MBD) and TMD. Pain, the most common symptom of TMD, was strongly associated with depression, anxiety and stress-related disorders, which are modified in TMD.[9] A prospective cohort study by Fillingim et al. identified several psychological variables as premorbid risk factors for initial TMD onset.[3] Meanwhile, Sójka et al.[4] and others[22–25] highlighted that comorbidity factors associated with TMD development include psychological stress, anxiety, emotional tension, and structural and parafunctional habits.

Several studies emphasize the importance of standardizing the assessment of psycho-emotional state performed by dentists and developing tools to refer patients for specialized consultation in the area of psycho-emotional disorders.[21–28]

If psycho-emotional disorders are believed to significantly contribute to TMD development, it is critical to diagnose them at the outset and to provide parallel support or psychiatric treatment within the TMD unit.[29,30] Seweryn et at. noted a correlation between the intensity of pain associated with TMD and the quality of life and sleep. These parameters were found to be influential in modifying TMD management.[31] Furthermore, Topaloglu-Ak et al. evaluated the relationship between sleep habits and TMD, bruxism and caries in children, revealing their potential negative impact on children's sleep habits and characteristics.[32]

A dentist's preliminary diagnosis should complement the specialized diagnosis of TMD and indicate further possible solutions. According to Yadav et al., stress is an important factor that is closely associated with problematic behaviors such as bruxism. Moreover, the authors found a significant correlation between this parafunctional activity and a higher degree of TMD symptoms.[18]

In view of the above, it is crucial to consider the necessity of psychological and/or psychiatric support in the course of TMD treatment.

Additionally, Martynowicz et al. emphasized that rhythmic masticatory muscle activity (RMMA) is a periodic muscle activity that characterizes sleep bruxism events. It can occur as a single event, in pairs or in clusters, and is connected with the severity of orofacial pain.[33]

During the implementation of the present project, the research results facilitated the decision on the indicated psychological or psychiatric consultation for patients treated for TMD. Moreover, the patients expressed their satisfaction with the recommendation of a necessary consultation regarding their psycho-emotional state.

The results of the study suggest that the developed questionnaire can be used as a supplement to the specialized examination for TMD diagnosis. In addition, it can assist dentists in identifying potential psycho-emotional issues in patients and recommending further therapeutic management.

Limitations

Since this is a preliminary report, validation of the questionnaire was not carried out. However, it is planned for the next stages of the project.

Conclusions

This research indicates that the developed questionnaire was significantly useful for conducting an initial assessment of the psycho-emotional state of patients during TMD diagnosis. Furthermore, the results highlight a greater need for psychological counseling in patients with TMD compared to healthy individuals.

Ethics approval and consent to participate

The study was approved by the Bioethics Committee of Jagiellonian University (consent No. 1072.6120.312.2021 – 15.12.2021; clinical trial No. 1072.6120.312.2021 – 15.12.2021; ID NCT06041633). Patients provided consent to participate in the research project.

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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