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Original paper

Impact of the COVID-19 pandemic on patients' anxiety levels related to dental appointments in Poland

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

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Abstract

Background. The reasons for the fear of coronavirus disease 2019 (COVID-19) infection are the ease of the transmission of the virus, the severe course of the disease and possible complications, as well as treatment difficulties. The dental office is a place of increased risk. Despite the applied epidemiological protection measures and the hygienic regimen, a dental visit may cause anxiety and generate stress.

Objectives. The aim of the study was to determine the level of fear of coronavirus infection in dental patients during the COVID-19 pandemic, taking into account the patients' age, gender and education, the number of people in the household, and the reason for the appointment. In addition, the patients assessed the epidemiological comfort provided during their visit to the dental clinic.

Material and methods. The survey was conducted among 100 adults who visited the University Dental Clinic (UKS) in Cracow, Poland, for dental treatment. The patients completed 2 questionnaires: "Assessment of the level of anxiety associated with COVID-19"; and "Impact of the COVID-19 pandemic on the need of dental treatment and the level of epidemiological safety of patients". The results were statistically analyzed and interpreted.

Results. The level of anxiety assessed with the questionnaire was moderate, and there were no statistically significant differences with regard to the age, gender and education of the respondents or the number of people in the same household. The main reason for reporting to the clinic was the desire to start and continue treatment. Most of the respondents believed that wearing a mask and measuring the temperature protect against infection, but 27% assessed the security measures as insufficient. Nearly half feared impeded access to dental services, and more than half were afraid of increased costs of treatment.

Conclusions. Despite the safety measures taken in place, patients felt anxious about dental appointments during the COVID-19 pandemic.

Keywords: anxiety, dental treatment, COVID-19, masks, epidemiological protection

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Introduction

Coronavirus disease 2019 (COVID-19) is an acute infectious disease of the respiratory system caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The most common symptoms of infection are a high fever, a dry cough, shortness of breath, muscle aches, fatigue, and loss of smell and taste. In most cases, infection takes a mild form, but it can proceed aggressively, leading to severe pneumonia, multi-organ failure, sepsis, a septic shock, and even death.[1–3]

The virus is transmitted via droplets, i.e., through sneezing and coughing. It is contracted through direct contact with a sick person, or by rubbing the eyes, nose or mouth with an infected hand.[4] The ease of pathogen transmission, non-specific symptoms and a long incubation period led to a worldwide epidemiological emergency a month after the first cases were diagnosed in the Chinese province of Hubei. In March 2020, the World Health Organization (WHO) granted COVID-19 a pandemic status, i.e., an epidemic on a global scale, and issued unified epidemiological recommendations.[5] Hygiene regimens, mouth and nose protection, and social isolation were the primary health protection methods, which could be modified by a given country depending on the number of patients and health service efficiency.[6] A surge in infections in the autumn and winter seasons in Poland forced the necessary introduction of orders and restrictions, resulting in changes in citizens' lives. To limit person-to-person contact, schools, offices, shopping centers, and sports and recreation venues were closed. In addition, organizing social meetings was forbidden and remote working was recommended. The ease of virus transmission, social isolation, and the sheer volume of information on the pandemic in the media and social networks affected mental health, and generated anxiety and depression.[7]

During the pandemic, access to healthcare was limited, which was mainly aimed at combating COVID-19. Teleconsultations were recommended for patient diagnostics and treatment to limit coronavirus transmission.[8] The planned procedures were postponed, and if in-person contact with a physician was essential, the visit followed epidemiological recommendations, i.e., maintaining a distance between the waiting patients, intervals between visits, and the protection of the mouth and nose of the patient and the doctor during the examination. The nature of some medical specialties hindered treatment, which could only be undertaken remotely or in compliance with sanitary requirements that limit viral transmission. One of such fields of medicine was dentistry. According to "The New York Times" report, it is one of the occupations most vulnerable to COVID-19 infection.[9] Indeed, the lack of a mask on the patient's face, the droplets of saliva or blood depositing on surfaces, and the inhalation of the aerosols generated by rotary and ultrasonic instruments all pose a threat to dental patients and dentists.[10] Therefore, new prophylactic and therapeutic protocols were introduced in medical facilities to protect medical personnel and patients during the pandemic.[11] Recommendations for patients included initial segregation through epidemiological interviews, temperature measurements, social distancing, and an obligation to cover the nose and mouth in public places.[12] The dental team was provided with protective clothing, gloves, visors, masks, and disinfectants.[13,14] However, despite an attempt to create optimal sanitary conditions for treatment, certain patients feared visiting the dentist, which could have worsened their oral health.

The study aimed to assess the feeling of anxiety caused by COVID-19 in dental patients during the pandemic with regard to the patients' age, gender and educational level, the number of people living in the same household, and the reason for reporting to the dental clinic, as well as to assess the patients' comfort with the preventive measures provided during their visit.

Material and methods

The study was approved by the Bioethics Committee at the Jagiellonian University, Cracow, Poland, on September 24, 2020 (No. 1072.6120.254.2020).

The research involved 100 patients aged 18–87 years who reported for general dental treatment to the University Dental Clinic (UKS) in Cracow, Poland, between October 21 and November 7, 2020. In the 1st week of the study, the daily increase in SARS-CoV-2 infections in Poland was 13,781 people, including 234 deaths, which was nearly doubled in the 2nd week (21,068 infected and 286 dead).

The research used 2 questionnaires. The 1st survey –"Assessment of the level of anxiety associated with COVID-19" – used the Fear of COVID-19 Scale (FCV-19S), developed by Ahorsu et al.,[15] which was adapted to Polish and validated. To the sheet consisting of 7 items, 4 items were added and summarized in the form of a table. The respondents marked their answers on a 5-point Likert scale, where '1' referred to 'strongly disagree' and '5' to 'strongly agree' (Appendix 1 available on request from the corresponding author). The maximum number of points that could be obtained was 55, and the minimum was 11. Scores of 11–25 points indicated a low sense of fear, 26–40 points demonstrated a moderate sense of fear, and 41–55 points meant a high sense of fear. Additionally, the patients subjectively rated their level of COVID-19 fear, using a numerical scale with scores from 1 to 10.

The 2nd questionnaire – "Impact of the COVID-19 pandemic on the need of dental treatment and the level of epidemiological safety of patients" – consisted of 19 closed

single-choice questions and collected sociodemographic data, i.e., age, gender, education, and the number of people in the household. The patients were asked about their reason for visiting UKS and the assessment of the epidemiological safeguards used (Appendix 2 available on request from the corresponding author). The questionnaire was distributed to patients registered for a visit by the UKS Central Registration Office employees. After reading the information about the study, the patients agreed to participate and consented for their data to be used for research purposes.

Statistical analysis

The study results were statistically analyzed using IBM SPSS Statistics for Windows, v. 29.0 (IBM Corp., Armonk, USA), and the Jamovi software, v. 2.3.28 (https://www.jamovi.org), which were employed to calculate basic descriptive statistics for quantitative variables and for other statistical analyses.

To verify the structure of the questionnaire, an exploratory factor analysis with Varimax rotation was performed, taking into account the Kaser–Mayer–Olkin (KMO) coefficient and Bartlett's test of sphericity. The age- and gender-dependent anxiety levels were compared using the multivariate analysis of variance (MANOVA). Pearson's χ^2 test and Fisher's exact test (the 2nd questionnaire) determined differences in the evaluation of epidemiological protective measures, face masks, temperature monitoring, treatment costs, as well as sociodemographic variables.

Results

A total of 46 males and 54 females aged 18–87 years participated in the study. The least numerous group consisted of 12 people aged below 29, and the largest was the senior group, which consisted of 38 people aged over 65. The educational level among the respondents was as follows: primary (n = 4); secondary (n = 44); vocational (n = 22); and higher (n = 30).

Most patients (n = 43) attended UKS to start treatment, 32 were interested in continuing treatment that started before the pandemic, and 25 experienced severe pain that required emergency outpatient intervention. After the symptoms disappeared, 9 people continued their treatment as planned.

The maximum number of points for responses to the individual questions of the 1st questionnaire was 50, and the minimum was 11. Seventeen participants reported low levels of anxiety related to the coronavirus pandemic, 70 reported moderate levels, and 13 reported high levels.

When asked about epidemiological safety during dental visits (the 2nd questionnaire), 13 males and 14 females believed that the applied preventive measures were insufficient. Meanwhile, 86 participants believed that wearing a mask protects other people, and 64 stated that measuring the temperature was sufficient for identifying infected individuals. Sixty-two respondents were concerned about the increased treatment costs caused by the additional protection required due to the pandemic. According to 19 males and 24 females, global coronavirus infections would result in impeded access to dental services. The youngest people (18–29 years of age) indicated that masks and temperature measurements were effective methods of protecting against the virus significantly less often than the oldest people (over 65 years of age). Those aged 30–50 years pointed to impeded access to dental services during the pandemic significantly more often than people aged 51–65 and over 65 years.

Evaluation of the results of the 1st questionnaire: "Assessment of the level of anxiety associated with COVID-19"

Assessment of the psychometric properties of the questionnaire

To verify the structure of the questionnaire, an exploratory factor analysis of principal components was performed using Varimax rotation. The KMO correlation coefficient was 0.86, and Bartlett's test of sphericity was statistically significant, which confirms the validity of distinguishing the factors. Based on the eigenvalues, 2 factors were isolated, which explained 61% of the variance in the anxiety levels. The scree plot confirmed the two-factor structure (Fig. 1).

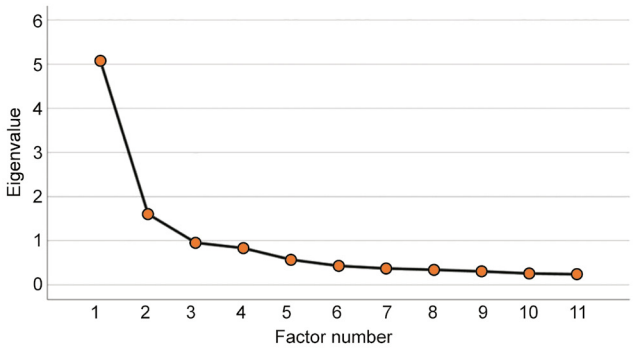

Fig. 1. Scree plot used in the exploratory factor analysis

Table 1 presents the matrix of the rotated components. The first 7 questions of the original scale constituted the 1st factor, and the additional questions were the 2nd one. Both factors had a satisfactory level of reliability of >0.8. The distribution of the respondents' answers to particular questions is presented in Fig. 2.

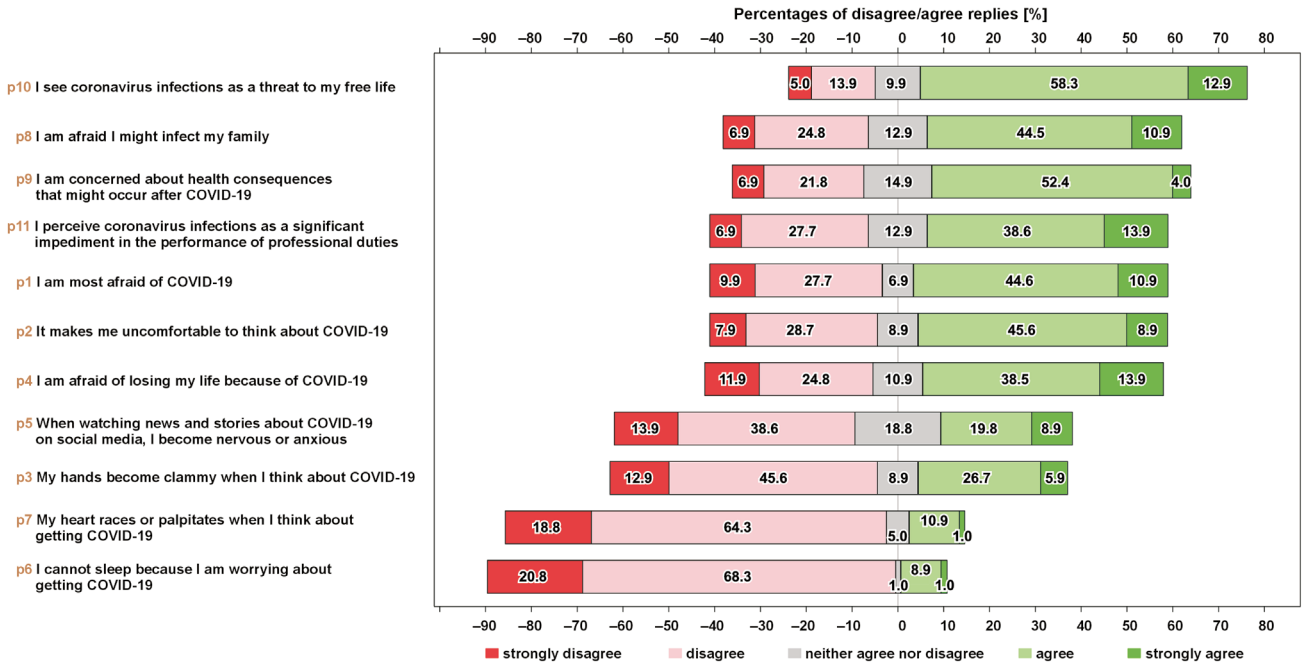

Fig. 2. Percentage distribution of the patients' responses to particular questions (Questionnaire 1: "Assessment of the level of anxiety associated with COVID-19") COVID-19 – coronavirus disease 2019.

Table 1. Matrix of the rotated components

Question	Factor	
	1	2
p1	**0.71**	0.21
p2	**0.81**	0.13
p3	**0.78**	0.24
p4	**0.73**	0.29
p5	**0.69**	0.28
p6	**0.74**	–0.03
p7	**0.62**	0.16
p8	0.26	**0.76**
p9	0.47	**0.65**
p10	0.22	**0.82**
p11	–0.01	**0.83**
Percentage of variance [%]	46.15	14.60
Reliability	0.868	0.816

Anxiety level and sociodemographic variables

To determine differences in the anxiety levels for factor 1 and factor 2 with regard to the patient's gender and age, the two-factor ANOVA was performed for many variables, with gender and age considered between-subject factors. The analysis showed no significant main effects for gender for factor 1 ($F(1.93) = 1.79$; $p = 0.185$; $\eta^2 = 0.02$) and factor 2 ($F(1.93) = 3.40$; $p = 0.069$; $\eta^2 = 0.04$). The main effects for age were also insignificant for factor 1 ($F(3.93) = 2.10$; $p = 0.106$; $\eta^2 = 0.06$) and factor 2 ($F(3.93) = 1.26$; $p = 0.293$; $\eta^2 = 0.04$). In addition, the interactions between the 2 between-subject factors were insignificant for factors 1 ($F(3.93) = 0.50$; $p = 0.682$; $\eta^2 = 0.02$) and 2 ($F(3.93) = 2.53$; $p = 0.062$; $\eta^2 = 0.08$). As such, the anxiety level was independent of the participant's gender and age.

The subjective assessment of the feeling of fear regarding COVID-19 was based on a numerical scale ranging from 1 to 10. The analysis of the correlation between this subjective assessment and factor 1 showed a strong positive relationship with anxiety ($r = 0.68$; $p < 0.001$), and a moderate positive relationship for factor 2 ($r = 0.47$; $p < 0.001$).

Evaluation of the results of the 2nd questionnaire: "Impact of the COVID-19 pandemic on the need of dental treatment and the level of epidemiological safety of patients"

The analysis showed no differences between genders in the assessments made. Detailed results are presented in Table 2. There were statistically significant differences between age groups in terms of opinions on protective masks, temperature measurements and access to treatment. The youngest subjects indicated that masks and temperature measurements were effective methods of preventing contact with people who could be a threat significantly less frequently than the oldest subjects. There were no differences between age groups in their assessment of epidemiological safeguards and treatment costs (Table 3).

Also, the educational level did not result in any differences in responses to the questionnaire. Regardless of the educational level, over 60% of respondents feared increased treatment costs (Table 4).

Table 2. Frequency analysis with Pearson's χ^2 test for the comparison of safety measures by gender

Safety measures	Gender		χ^2	*p*-value	φ
	male *n* = 46	female *n* = 54			
Epidemiological safeguards	14 (30.4)	13 (24.1)	0.59	0.442	0.08
Protective mask	40 (87.0)	46 (85.2)	0.22	0.640	0.05
Temperature measurement	31 (67.4)	33 (61.1)	0.59	0.443	0.08
Treatment costs	27 (58.7)	35 (64.8)	0.26	0.612	0.05
Access to treatment	24 (52.2)	19 (35.2)	3.18	0.074	0.18

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

Table 3. Frequency analysis with Fisher's exact test for the comparison of safety measures by age

Safety measures	Age [years]				*p*-value	*V*
	18–29 *n* = 12	30–50 *n* = 24	51–65 *n* = 27	>65 *n* = 37		
Epidemiological safeguards	4 (33.3)	6 (25.0)	6 (22.2)	11 (29.7)	0.876	0.08
Protective mask	7 (58.3)[a]	19 (79.2)[a,b]	23 (85.2)[a,b]	37 (100.0)[b]	0.005*	0.35
Temperature measurement	4 (33.3)[a]	12 (50.0)[a,b]	18 (66.7)[a,b]	30 (81.1)[b]	0.014*	0.32
Treatment costs	9 (75.0)	19 (79.2)	14 (51.9)	20 (54.1)	0.098	0.25
Access to treatment	5 (41.7)[a,b]	17 (70.8)[b]	8 (29.6)[a]	13 (35.1)[a]	0.013*	0.33

Data presented as *n* (%). * statistically significant; values with different superscript letters differ at $p < 0.05$.

Table 4. Frequency analysis with Fisher's exact test for the comparison of safety measures by the educational level

Safety measures	Educational level				*p*-value	*V*
	primary *n* = 4	secondary *n* = 44	vocational *n* = 22	higher *n* = 30		
Epidemiological safeguards	1 (25.0)	14 (31.8)	4 (18.2)	8 (26.7)	0.718	0.12
Protective mask	3 (75.0)	38 (86.4)	19 (86.4)	26 (86.7)	0.463	0.16
Temperature measurement	3 (75.0)	29 (65.9)	16 (72.7)	16 (53.3)	0.519	0.15
Treatment costs	3 (75.0)	27 (61.4)	16 (63.6)	18 (60.0)	1.000	0.03
Access to treatment	2 (50.0)	17 (38.6)	6 (27.3)	18 (60.0)	0.104	0.25

Data presented as *n* (%).

Discussion

Scientific research demonstrates the negative impact of the COVID-19 pandemic on mental health globally.[15–17] The fear of coronavirus infection, the socio-economic impact of the pandemic, the persistent tracking of information in the media and social networks, xenophobia, and traumatic thoughts about the disease have been defined by scientists in Canada and the United States as COVID Stress Syndrome.[18] The severe course of the disease, hospitalization, health complications, and the death of relatives may lead to post-traumatic stress, depression and anxiety disorders. Many tools have been developed to analyze the psycho-emotional state of society during the COVID-19 pandemic in more detail, including surveys and questionnaires, which have also been adapted to the Polish language. These include FCV-19S, the Obsession with COVID-19 Scale (OCS), the COVID Stress Scale (CSS), and others routinely used in psychological research.[15,18–20]

The current study used the FCV-19S questionnaire, to which 4 statements were added to cover the socio-economic aspect of the pandemic, i.e., material effects, and changes in family and professional life that may negatively impact the patient's psychological comfort. After statistical analysis, the questionnaire was deemed a reliable psychometric tool, and showed that the fear of COVID-19 was low, moderate or high, and similar to the level assessed by the respondents on a scale from 1 to 10.

The results show that the level of fear related to coronavirus was independent of the respondent's age. Similar to our research, a meta-analysis of data collected from 10 countries found the lowest level of anxiety in the youth and the elderly.[21] This finding is explained by a lower awareness of the real threat of the pandemic amongst young people, and reconciliation with fate and the passage of time in the elderly.[22] A different relationship was observed by researchers from Italy,[22] China,[23] Japan,[24] Turkey,[25] and Poland,[7] who showed that the anxiety levels

increased with age. The reason for this can be found in the more severe course of viremia, a worse prognosis and numerous complications correlated with the burden of comorbidities in the elderly.

Comparing the level of fear of COVID-19 between males and females did not show statistically significant differences, though it was higher in women. This result confirms the hypothesis put forward by Tolin and Foa,[26] and supported by many studies, stating that females have increased sensitivity to stress and react more strongly to pandemic threats.[26,27] Susceptibility to stressful situations also depends on the marital status, education, and family and professional situations. Indeed, surveys conducted among the Indian population demonstrated a greater reactivity to stress in married people, those with a lower educational level and individuals working in the health service.[28] However, this was not observed in the current study, although the presence of stress makes it possible to use FCV-19S to identify the occupations and professions most exposed to infection and stress.

Regardless of gender, 86% of the respondents believed that mask-wearing protects against SARS-CoV-2 infection, with the elderly being the largest percentage. Over 60% assessed that the temperature measurement and the pre-visit interview eliminated the potential risk. Besides the possibility of coronavirus infection, the patients visiting the clinic during the pandemic were most concerned about increased treatment costs and limited access to healthcare. Being aware of the possibility of contracting the virus during the procedure and while waiting, and transmitting it to their relatives, they mainly attended the clinic to start treatment or receive emergency pain relief. The fear of COVID-19 and the phobias caused by the traumatic nature of dental work contribute to the cancelations of dental visits. A survey conducted in Turkey showed that 73% of participants feared dental treatment because of the possibility of contracting the virus in the dental office.[29] Moreover, the literature reports that the likelihood of treatment discontinuation is 6 times higher in people with a high level of fear of coronavirus and 8 times higher in the elderly.[14] Almost 25% of respondents changed their treatment date during the periods of increased infection rate due to the possibility of infection.[14] These findings differ from an analysis carried out in Madrid, Spain, which showed that over 90% of those asked would go to the dentist willingly despite the risk.[30]

Conclusions

The state of the pandemic and concerns about contracting SARS-CoV-2 caused fear in dental patients. Despite the restrictions introduced and attempts made to create optimal sanitary conditions, the fear of infection reduces patient confidence in medical staff, which may result in dental visit postponement and worsen oral cavity health.

Ethics approval and consent to participate

The study was approved by the Bioethics Committee at the Jagiellonian University, Cracow, Poland, on September 24, 2020 (No. 1072.6120.254.2020). The informed written consent was obtained from all the participants.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Instantaneous dental implant loading technique by fixed dentures: A retrospective cohort study

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Abstract

Background. In the context of dental prostheses, splinting multiple implants together may improve their stability. The approach may be especially favorable when performing immediate loading procedures, increasing the implant osseointegration rate, and reducing the risk of implant and prosthetic failure. The instantaneous loading technique (ILT) involves creating a metal framework to splint the implants by intraorally welding them pair-by-pair, using purposefully created abutments.

Objectives. The aim of the study was to investigate the prosthetic success when using ILT to rehabilitate partially edentulous patients through immediately loaded prostheses.

Material and methods. Clinical records of patients treated with ILT were retrospectively assessed, and the prosthetic success rate was analyzed in terms of fractures, chipping, unscrewing, screw fracture rate, and mucositis. Furthermore, the implant success rates were evaluated by measuring marginal bone loss (MBL).

Results. A total of 55 patients (20 males and 35 females with a mean age of 59.8 ±9.4 years), corresponding to 66 prostheses, were included. A total of 160 implants were placed. At the last follow-up (39.6 ±28.4 months), 1 patient (1.8%; 1 prosthesis (1.5%)) showed the fracture of the prosthesis material. Peri-implantitis affected 4 implants (2.5%), and 4 more implants (2.5%) showed radiolucency, affecting 5 patients (9.1%). Two other patients (3.6%) suffered from mucositis. The implant success rate, according to the Albrektsson and Zarb criteria, was 94.4%. No implants were lost. The mean MBL values at the implant level, the prosthesis level and the patient level were 0.28 ±0.56 mm, 0.30 ±0.51 mm and 0.33 ±0.54 mm, respectively.

Conclusions. The instantaneous loading technique appears to be a viable approach to rehabilitating partially edentulous patients through immediate loading.

Keywords: jaw, edentulous, denture, dental prosthesis, implant-supported

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Introduction

Immediate loading refers to delivering the prosthesis and allowing masticatory load within 48 h of implant placement,[1] without waiting for osseointegration, as initially proposed by Brånemark.[2,3] The benefits of immediate loading include immediate functional rehabilitation, and reduced discomfort and morbidity. Under appropriate conditions, the implant and prosthetic success rates of immediate loading are not significantly different from those of the delayed approach.[3–5]

For immediate loading to be successful, fixtures, as in delayed loading, must not undergo micro-movements exceeding 100–150 μm. If this occurs, fibrous tissue may develop at the bone–implant interface, leading to implant failure.[3,6] Therefore, implants must show adequate primary and secondary stability. Several strategies have been proposed to improve implant stability and soft tissue healing around dental implants. For instance, photobiomodulation, a non-invasive, non-thermal therapy capable of stimulating cellular self-repair, has demonstrated encouraging results, especially during the early stages of healing.[7]

Along with implant stability, raising the awareness of the biochemical aspects of peri-implant tissue should be pursued. Indeed, a more severe pro-inflammatory state has recently been associated with peri-implant tissue as compared to periodontal tissue.[8–10] Greater inflammation seems to be due to prosthetic components per se and to their micro-movements, as well as to the presence of excessive cement in cement-retained implant restorations.[11–13]

As a further precaution, prostheses should be designed and connected to implants such that no residual tensions are transferred to the bone–implant interface,[3,14–16] which will also reduce complications, such as abutment fracturing, or screw breaking or loosening (in the case of screwed-on prostheses).[17,18] To this aim, splinting the abutments increases their mechanical stability and allows more accurate impressions.[19–22] Accordingly, some authors have proposed, especially for full-arch rehabilitation, to splint all abutments through a single titanium bar, which is initially modeled to fit the abutments, followed by welding.[20,21] However, modeling the bar is relatively complex, and fitting it to all abutments with no residual tension involves a painstaking trial and error procedure, as adjustments made to one position may create a residual tension elsewhere along the bar.[20]

To overcome these issues, we devised an approach consisting in splinting the adjacent abutments pair-by-pair. Splinting the abutments in pairs eliminates passive tensions, and the complete structure connecting all abutments displays no tension. This is achieved using special abutments (Wings®; T.A.B., Borso del Grappa, Italy) featuring lateral extensions (Fig. 1). The abutments are initially screwed to the implants and their extensions are welded intraorally to solidarize the two adjacent implants. The final metal framework acts as the internal reinforcement structure of the provisional prosthesis fabricated and delivered to the patient on the same day. We named this approach the instantaneous dental implant loading technique, abbreviated to the instantaneous loading technique (ILT), and have been using it for approx. 20 years.

Despite the long-term use of ILT, its clinical outcomes have never been thoroughly studied. Therefore, this study aimed to retrospectively investigate the medium-term frequency of prosthetic complications following the application of ILT to rehabilitate partially edentulous patients.

Material and methods

Clinical investigation

This clinical investigation involved the retrospective assessment of the clinical records of patients who presented with partial edentulism and were rehabilitated using ILT between March 2003 and August 2020 at an Italian private dental clinic in Noventa Vicentina. The Internal Ethics Commission of the clinic assessed and approved the study protocol. Given the retrospective nature of the study, the Commission did not deem it necessary to seek the approval of a third-party ethics committee. The patients' records were selected according to the following inclusion criteria: age between 18 and 85 years; rehabilitation with the use of ILT for partial edentulism; no previous bone graft or bone regeneration intervention; no previous peri-implant bone regeneration technique applied; the details of an immediate intraoral radiograph after implant insertion, taken with a Rinn centering device and a customized silicone bite; a follow-up of at least 6 months after implant placement; at least 1 intraoral radiograph carried out at the last follow-up visit, again with a Rinn centering device and a customized silicone bite; and evidence of informed consent for the use of their clinical data for future clinical investigations. The clinical records of the patients were discarded if any of the following exclusion criteria were met: suffering from osteoporosis or other bone diseases; undergoing bisphosphonate therapy; suffering from any psychiatric disorder or neoplasia; pregnancy; a history of chemotherapy or radiotherapy in the head or neck region at any time in the previous 2 years; immunocompromised; suffering from acute oral infections and/or coagulation disorders; a history of alcohol or drug abuse; smokers; and taking any drug known to interfere with the osseointegration process. The patients could have implants of any brand, but had to have received the provisional or final prosthesis made of the same materials, and manufactured by the same dental technician.

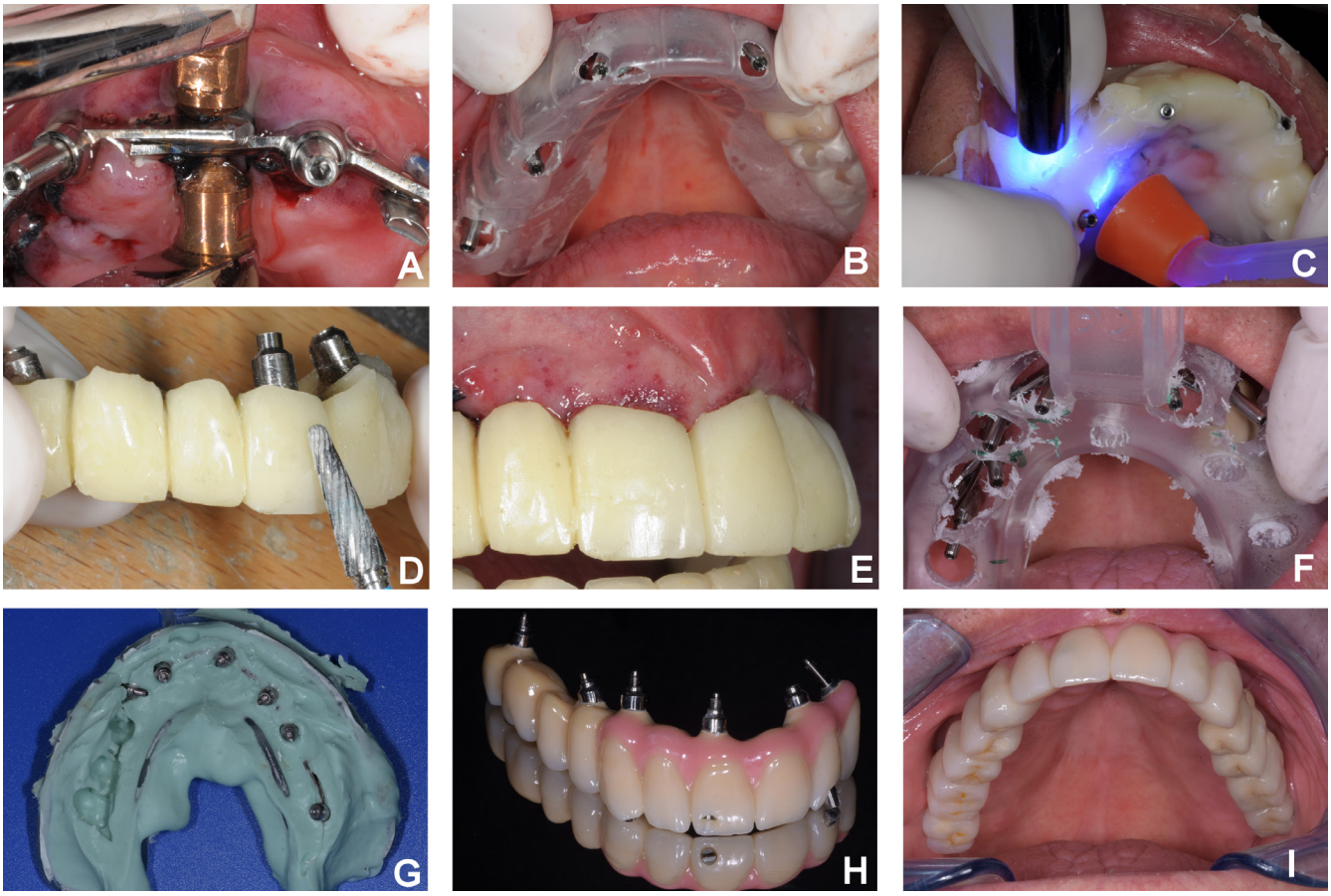

Fig. 1. Main steps of the instantaneous loading technique (ILT)

A – the extensions of the adjacent wing abutments are welded; B – a thermoplastic prosthetic template is positioned in the mouth and drilled correspondingly to the abutments; C – after filling the template with a photopolymerizing resin, the resin is photopolymerized to fabricate the provisional prosthesis; D – the provisional prosthesis is finished by the technician; E – the provisional prosthesis is delivered to the patient; F – to fabricate the final prosthesis, another wing structure is welded and an impression is taken with the use of a tray purposefully drilled correspondingly to the abutments; G – the metal framework is embedded in the impression; H – the impression is used to fabricate the final prosthesis; I – the final prosthesis is delivered to the patient.

All implants needed to have been placed using a personalized surgical guide based on the flapless technique, and the implant seat preparation had to have been carried out according to the manufacturer's protocol.

Surgical and implant placement protocol

After clinical examination and radiographic assessment based on intraoral radiographs and cone-beam computed tomography (CBCT), the diameters, lengths and positions of the implants were pre-planned on the CBCT scans, and a surgical guide was manufactured. Antibiotic prophylaxis (2 g amoxicillin/clavulanic acid (Augmentin); GlaxoSmithKline, Verona, Italy) was prescribed 1 h before surgery, and then every 12 h for 8–10 days. The patients were also advised to carry out mouth rinses with 0.2% chlorhexidine (Corsodyl; GlaxoSmithKline, London, UK) for up to 2 weeks after surgery. Naproxen (500 mg) (Synflex, Milano, Italy) was prescribed 2–4 times a day for 7 days after surgery for pain control.

The surgical area was anesthetized using articaine hydrochloride (40 mg/mL) with epinephrine (1:100,000) (Citocartin, Milan, Italy). With the aid of the guide, the implant placement positions were identified using a dermographic pen, commonly employed to mark the anatomical landmarks/positions of the human body. Access to the alveolar bone was achieved with a mucotome, and the implants were placed according to the manufacturer's instructions with the aid of the guide. The patient was then rehabilitated using ILT (see the following paragraph). Radiographic assessment with the use of intraoral radiographs was performed before surgery, at the provisional (immediately) and final (6 months later) prosthesis delivery, and at least every 12 months thereafter.

Instantaneous loading technique

The instantaneous loading technique involves fabricating the provisional or final prosthesis over a metal structure composed of several wing abutments welded to each

other to connect the adjacent implants (Fig. 1). Wing abutments are available in different lengths (1.7 mm, 2.7 mm and 4.5 mm). The abutments have different angles (flat, 30° or 45°) and all feature two 11.5-millimeter-wide lateral extensions (i.e., the 2 'wings') that can be cut to the desired length before welding. The abutments are connected to the implants with 20-millimeter-long pass-through screws. The extensions of the 2 adjacent wing abutments are overlapped, and then welded. The extensions are flat on the vestibular side and convex on the buccal side, which limits the contact surface between the 2 wings. Delivering a certain welding current increases electrical resistance, and the heat which develops at the contact point creates a strong and resistant weld. To deliver the provisional prosthesis, the technician fabricates a prosthetic template made of a thermoplastic material based on the diagnostic waxing from the alginate impression of the arch of interest. The template is then positioned on the welded wing abutment framework in the oral cavity and drilled correspondingly to the pass-through screws, using a diamond bur. The template is then filled with a photopolymerizing resin, with the screws protruding from the template, and the resin is photopolymerized. The prosthesis is then screwed to the metal structure. A new wing structure is created after 6 months as the armor of the final prosthesis. An alginate impression of the metal structure is obtained, with holes at the positions corresponding to the pass-through screws. The metal structure is then sent to the technician, who utilizes it to fabricate the final prosthesis using a composite resin. The prosthesis is then implanted by tightening the screws at 35 N·cm. The screw holes are filled with the same composite resin as used to manufacture the prosthesis. A representative case is shown in Fig. 2.

Objectives and endpoints

The primary objective of this study was to assess the prosthetic success of definitive rehabilitation delivered through ILT by evaluating prosthesis fractures, chipping, unscrewing, screw fractures, mucositis, and peri-implantitis. The secondary objective was to assess the implant success and survival when supporting the prostheses delivered through ILT by measuring marginal bone loss (MBL) and using the criteria of Buser et al.,[23] modified by Albrektsson and Zarb.[24,25] The criteria were as follows: the absence of persistent pain, dysesthesia or paresthesia in the implant area; the absence of peri-implant infection, with or without suppuration; the absence of perceptible implant mobility; and the absence of more than 1.5 mm of peri-implant bone resorption during the first year of loading or 0.2 mm of resorption per year during the following years. The implants were considered successful if they met all the conditions outlined above.

Measurement of marginal bone loss

For all the included records, the intraoral radiographs were digitally scanned, converted to 600 dpi resolution TIFF images, stored in a personal computer, and analyzed with the ImageJ software (National Institutes of Health, Bethesda, USA) to measure the peri-implant MBL. After loading each image, the software was calibrated using the known implant diameter at the most coronal portion of the implant neck. The distance from the implant–abutment interface to the most apical point of the crestal bone in intimate contact with the implant was then measured to the nearest 0.01 mm on both the mesial and distal sides. The 2 measurements were averaged to get a single peri-implant marginal bone level. Then, at the final follow-up visit, MBL for the particluar implant was calculated as the difference between the peri-implant bone level at that time point and that at baseline (at implant insertion).

The mean MBL was assessed at the implant level, the prosthesis level and the patient level. In the first case, the mean MBL was calculated by averaging all MBL values for all implants. At the prosthesis level, the mean MBL was calculated as the average of the MBL values for all the implants supporting the same prosthesis. At the patient level, the mean MBL for each patient was calculated by averaging the MBL values for all his/her implants.

Bias

Since the patients were treated by only one of the authors (S.D.), which represents a possible source of bias, clinical record selection and data extraction were carried out by the other 3 authors (M.M., A.G. and N.Z.). As a further strategy to address the potential sources of bias, an independent biostatistician performed all statistical analyses.

Statistical analysis

Since the study objective was to investigate the prosthetic success of ILT using descriptive statistics, no sample size calculation was performed. Therefore, the population size of the study corresponds to the number of records meeting the inclusion criteria.

The descriptive statistics of the patient's age and sex, the follow-up duration, and the complication rates were collated using the patient as the statistical unit of analysis. Further descriptive analyses, including the calculation of the complication rates, were carried out using the implants and the prostheses as the statistical analysis units. All statistical analyses employed the Origin 2021 software (Microcal Software Inc., Northhampton, USA). Statistical tests were regarded as significant at $p < 0.05$. The results were expressed as mean and standard deviation ($M \pm SD$) or as median and interquartile range (Me (IQR)) depending on whether their distribution was normal or non-normal, respectively, according to the Shapiro–Wilk test.

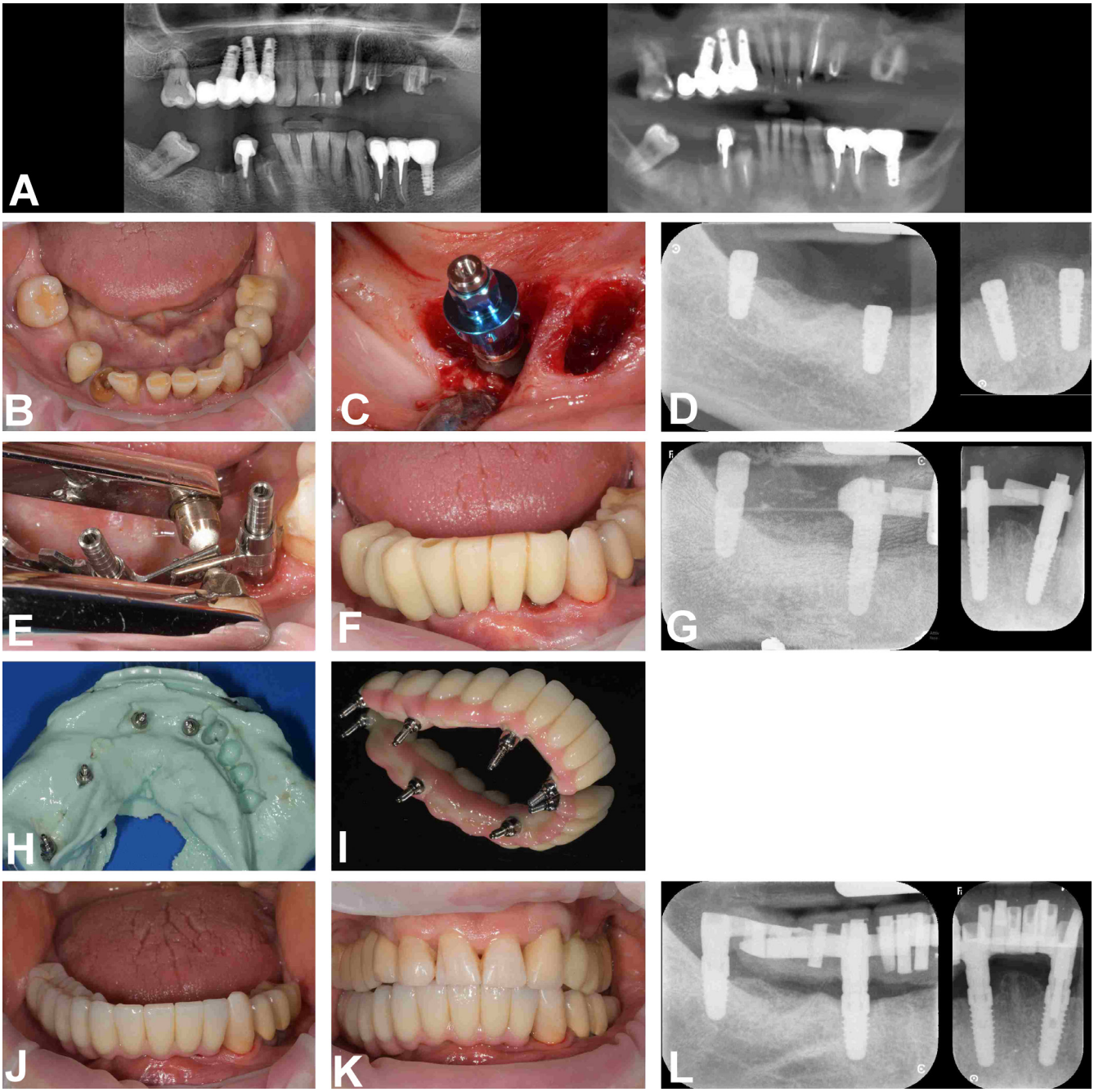

Fig. 2. Illustrative case, showing the application of the instantaneous loading technique (ILT) to fabricate a four-implant, nine-crown prosthesis

The patient at presentation was partially edentulous in the 4th quadrant (A,B); teeth 3.2, 3.1, 4.1, 4.2, 4.3, 4.4, 4.7, as well as the 4.5 residual root, were lost and extracted. Four implants were placed according to a flapless approach (C,D). After positioning the wing abutments, their extensions were welded (E) and an impression was taken, embedding the framework, and the provisional prosthesis was delivered to the patient (F,G). To fabricate the final prosthesis, a new wing framework was created 6 months later and an impression was taken (H), embedding the framework, which was used to fabricate the final prosthesis (I) to be delivered to the patient (J,K,L). The image panels show the intraoral radiographs taken at implant insertion (D), the provisional prosthesis delivery (G) and after 18 months of prosthetic rehabilitation (L).

Results

In all, 55 patients with clinical records that met the study criteria were included. These comprised 20 males and 35 females with a mean age at surgery of 59.8 ±9.4 years, ranging from 41 to 82 years. The patients were supplied with 160 implants and 66 prostheses. Forty-eight patients received 1 prosthesis, 4 received 2 prostheses, 2 received 3 prostheses, and 1 received 4 prostheses. The replaced elements, the number of implanted prostheses and the implant manufacturer are listed for each patient in Table 1. The distribution of prostheses in the arches is reported in Table 2. The prostheses replaced 2–9 lost elements (Table 3). Seventy-two (45.0%) and 88 (55.0%) implants were inserted in the maxilla and the mandible, respectively. While the implants differed in size

Table 1. For each patient, the replaced elements, the number of implanted prostheses and the implant manufacturer are reported

Patient	Replaced elements*	Number of implanted prostheses	Implant manufacturer
1	29, 30, 31	1	Biomax, Vicenza, Italy
2	30, 31	1	BTK, Dueville, Italy
3	30, 31	1	iRES®, Mendrisio, Switzerland
4	4, 2	1	BTK, Dueville, Italy
5	20, 31	1	BTK, Dueville, Italy
6	2, 4, 5	1	BTK, Dueville, Italy
7	28, 30	1	BTK, Dueville, Italy
8	2, 3, 10, 13, 15, 30, 31	3	BTK, Dueville, Italy
9	29, 31	1	Biomax, Vicenza, Italy
10	11, 13, 15	1	Biomax, Vicenza, Italy
11	12, 13, 14, 15	1	BTK, Dueville, Italy
12	28, 29, 31	1	iRES®, Mendrisio, Switzerland
13	17, 20	1	Biomax, Vicenza, Italy
14	2, 5	1	BTK, Dueville, Italy
15	3, 4	1	BTK, Dueville, Italy
16	18, 20	1	BTK, Dueville, Italy
17	29, 31	1	BTK, Dueville, Italy
18	30, 31	1	Biomax, Vicenza, Italy
19	12, 14, 15	1	BTK, Dueville, Italy
20	18, 20	1	BTK, Dueville, Italy
21	18, 19	1	BTK, Dueville, Italy
22	7, 10	1	JDentalCare, Modena, Italy
23	18, 19	1	Biotech Dental Italia, Salerno, Italy
24	29, 31	1	iRES®, Mendrisio, Switzerland
25	23, 26	1	JDentalCare, Modena, Italy
26	30, 31	1	iRES®, Mendrisio, Switzerland
27	18, 20	1	BTK, Dueville, Italy
28	2, 3, 4	1	BTK, Dueville, Italy
29	18, 19	1	BTK, Dueville, Italy
30	18, 19	1	BTK, Dueville, Italy
31	30, 31	1	BTK, Dueville, Italy
32	19, 21	1	BTK, Dueville, Italy
33	13, 14, 15	1	BTK, Dueville, Italy
34	12, 14, 15	1	BTK, Dueville, Italy
35	18, 19	1	BTK, Dueville, Italy
36	23, 26	1	BTK, Dueville, Italy
37	10, 12, 13, 15	1	Biomax, Vicenza, Italy
38	18, 19	1	iRES®, Mendrisio, Switzerland
39	18, 19, 21, 27, 29, 31	2	Biomax, Vicenza, Italy
40	30, 31	1	Biotech Dental Italia, Salerno, Italy
41	2, 4	1	Biomax, Vicenza, Italy
42	30, 31	1	BTK, Dueville, Italy
43	2, 3, 5, 7, 9, 11	1	BTK, Dueville, Italy
44	30, 31	1	BTK, Dueville, Italy
45	18, 20 ,30, 31	2	BTK, Dueville, Italy
46	2, 3, 5, 7, 8, 12, 13, 15	3	BTK, Dueville, Italy
47	13, 15	1	BTK, Dueville, Italy
48	18, 19	1	Biomax, Vicenza, Italy
49	1, 5, 6, 11, 12, 15, 18, 19, 28, 30, 31	4	BTK, Dueville, Italy
50	7, 6, 12, 13, 15	2	BTK, Dueville, Italy
51	18, 19, 20, 30, 31	2	BTK, Dueville, Italy
52	18, 19	1	BTK, Dueville, Italy
53	14, 15	1	BTK, Dueville, Italy
54	23, 25, 29, 31	1	BTK, Dueville, Italy
55	14, 15	1	BTK, Dueville, Italy

* American numbering system.

Table 2. Distribution of prostheses according to their position in the patients' mouth

Prosthesis position	Number of prostheses
Upper right	14
Upper central	1
Upper left	11
Lower right	21
Lower central	2
Lower left	17
Total	66

Table 3. Distribution of prostheses according to the number of elements they replaced

Number of elements replaced by the prosthesis	Number of prostheses
2	26
3	18
4	14
5	3
6	4
9	1
Total	66

(the diameters and lengths were 3.25, 3.50, 3.70, 3.75, 4.00 mm, and 8.5, 10.0, 10.5, 11.5, 12.0, 13.0, 15.0 mm, respectively), they were all tapered in shape and had a double-etched, sandblasted surface. The average follow-up time from implant insertion and loading (loading was immediate) was 39.6 ±28.4 months (range: 7–213 months; *Me* (*IQR*): 35 (26–50) months). A total of 178 intraoral radiographs were analyzed.

After implant placement, all patients healed uneventfully, with no reports of subjective complaints. Technical complications concerned 1 prosthesis (1.5%) and 1 patient (1.8%), in whom the composite material of a single prosthesis fractured. The prosthesis was replaced with a provisional one stored at the clinic and sent to the technician for repair. No prosthetic frames were fractured and no prostheses became unscrewed. Furthermore, neither the screws nor the implants were fractured. Peri-implantitis affected 2 patients (3.6%) and 4 implants (2.5%), with 2 implants per patient. Four implants (2.5%) in 3 patients (5.5%) (2 patients had 1 implant each, and the third patient had 2 implants) showed peri-implant radiolucency. Two patients (3.6%) suffered from mucositis.

Overall, 151 (94.4%) implants were successful, according to the Buser et al.[23] and modified Albrektsson and Zarb criteria.[24,25] At the last follow-up control, there were 9 implants (5.6%) that survived and no implant was lost.

At the implant level, the average marginal bone resorption at the last follow-up was 0.28 ±0.56 mm (range: 0.00–3.15 mm). Meanwhile, it was 0.30 ±0.51 mm (range: 0.00–2.74 mm) at the prosthesis level and 0.33 ±0.54 mm (range: 0.00–2.74 mm) at the patient level.

Discussion

Our results show that over a medium-term follow-up, ILT allows the successful rehabilitation of partially edentulous patients by delivering a provisional prosthesis that can be immediately loaded. The observed technical and biological complication rates are consistent with similar approaches involving splinting implants through a single bar.

In a study on 40 patients rehabilitated by immediately loaded provisional prostheses built over a continuous titanium bar welded intraorally, Degidi et al. in 2006 first reported a prosthesis success rate of 100% over 6 months after placement.[20] These results were similar to ours. Our results can also be compared to those reported by Albiero et al., who observed no mechanical or biological complications at a 1-year follow-up in 10 consecutive patients rehabilitated using 60 immediately loaded implants supporting fixed full-arch prostheses constructed over intraorally welded bars and placed through a computer-guided flapless procedure.[26] In 2015, Marchesi et al. achieved similar results over 26.5 months, after rehabilitating 17 consecutive patients with 2 parallel and 2 tilted implants in the maxilla, splinting their angulated abutments with an intraorally welded titanium bar.[27] Similar results were also reported by Degidi et al., who prospectively evaluated 40 patients with an edentulous mandible, using a fixed restoration supported by an intraorally welded titanium bar placed on the same day as the implants; they reported no evidence of framework fractures at a 24-month follow-up.[21] Meanwhile, Degidi et al. prospectively treated 20 patients with 4 interforaminal implants that were immediately loaded with a fixed restoration supported by an intraorally welded titanium framework, and observed no fractures or radiographically detectable alterations of the welded frameworks 24 months after surgery.[28] The same group also prospectively assessed 30 patients who received 3 axial and 4 tilted implants in the edentulous maxilla.[29] Immediately after implant placement, the definitive abutments were connected to the implants and a titanium bar was welded to them with an intraoral welding unit. This framework supported the definitive restoration. At 36 months, the authors observed no fractures or radiographically detectable alterations of the welded frameworks.[29] Degidi et al. prospectively treated 60 patients with fully edentulous arches with 324 immediately loaded implants and delivered fixed restorations supported by intraorally welded titanium bars.[30] At a 36-month follow-up, they recorded no fractures or radiographically detectable alterations of the welded frameworks.[30]

The MBL observed in our study cannot be directly compared with the values reported previously, given the different nature of the surgical and prosthetic protocols involved. Overall, the implant success rates and the mean MBL observed in the present study appear consistent with those generally noted in other immediate loading rehabilitation procedures.[31,32] Therefore, the current research supports the working hypothesis that the core of ILT, namely splinting the adjacent implants in pairs, promotes excellent passivation, and limits complications in the implants and the prostheses they support. The authors, based on their experience, found ILT easier and faster than any approach they had tried involving the welding of a single titanium bar to splint more implants together. The technique is also made easier by the different angles of extensions with which wing abutments are provided, which was particularly helpful and straightforward when creating a complete metal framework. These observations should be the subject of comparative studies assessing if the learning curve and the surgery time differ between the 2 techniques (wing abutments/a single bar) under homogenous conditions. To the best of our knowledge, and as outlined by other authors using similar approaches, the only contraindication for this technique that could increase the rate of prosthesis fractures seems to be bruxism.[33,34] Finally, delivering a prosthesis based on ILT involves less costs for the patient than other rehabilitation methods and may be an option for those who cannot afford more expensive treatment.

Limitations

A limitation of the present study was its retrospective design. Therefore, prospective studies should assess the performance and safety of the ILT approach. Such studies may reduce the possibility of confounding variables affecting the results and may enable the evaluation of specific covariates, other than the technique itself, that modulate the technical and biological complications and MBL when patients are rehabilitated via this approach. Moreover, future studies should use the same implants rather than various devices like in the present study. Comparative studies should assess whether ILT provides any advantage over other techniques involving the splinting of the adjacent implants by intraoral welding and the use of a framework to support an immediately loaded restoration. Patients should be followed up for a longer time to validate the present results.

Conclusions

Within the limitations of the present study, ILT seems a viable approach to rehabilitating partially edentulous patients through immediate loading. Whether ILT is easier, faster, and as safe and effective as other technical approaches involving the intraoral welding of a bar connecting all implants should be the subject of appropriately designed prospective, comparative clinical studies.

Ethics approval and consent to participate

The study protocol was assessed and approved by the Internal Ethics Commission of a private dental clinic in Noventa Vicentina, Italy. The patients had given their informed consent for the use of their clinical data for future clinical investigations (one of the inclusion criteria).

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

Design of customized coated dental implants using finite element analysis

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Abstract

Background. Dental implants are used as a traditional technique to replace missing teeth. In the long-term evaluation of dental implants, the stability and durability of the implant–bone interface are crucial. Furthermore, the success of dental implants depends on several factors, such as osseointegration, implant geometry and surface topography.

Objectives. The aim of the study was to investigate the effects of coating materials on dental implants by altering several parameters, including the material used, the coating thickness, and different combinations of the cortical and cancellous bones.

Material and methods. The coating materials used were hydroxyapatite (HAP), monticellite (MTC) and titanium nitride (TiN). The coating thickness was varied as 50 µm, 100 µm, 150 µm, and 200 µm. Five different bone combinations were used for the proposed finite element model. An axial compressive load of 150 N was applied.

Results. The FEA showed that the HAP coating material had a significant effect on minimizing the induced stress concentration for all 5 bone combinations. However, the MTC coating material had a significant effect only on 2 bone combinations (combination 2 and combination 3). Meanwhile, the TiN coating material induced higher stress values.

Conclusions. Based on finite element analysis (FEA), it was observed that the coating thickness greatly influenced the concentration of the mechanical stress. Indeed, when the coating thickness was relatively high, the stress concentration value significantly decreased.

Keywords: bone condition, finite element analysis, dental implant, coating thickness, coating materials

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Introduction

Dental implants have become a traditional technique used for replacing missing teeth in recent decades.[1] The success of dental implants, in both the short and long term, depends upon osseointegration and other factors, such as implant geometry and surface topography.[2,3] For their long-term success, dental implants need improved surface characteristics and biocompatible materials, which have better mechanical properties than commercially pure titanium (Ti), including alloys such as Grade 5 titanium (Ti-6Al-4V) and titanium-zirconium (Ti-Zr).[4]

There is some evidence regarding the short-term failure of implants due to biological and biomaterial-related factors as well as surface treatment.[5–8] Other factors, such as stress shielding and implant loosening, may directly reduce the longevity of implants and necessitate frequent revision surgeries.[9] Furthermore, corrosion can cause extensive implant fatigue, which may shorten the life of the device and lead to its failure. In this context, the most commonly used Ti as well as Ti alloys lack the stability required to prevent corrosion in the initial stages post-implantation.[10,11] Indeed, an investigation on the functionalization of the surface at the bone–implant interface has demonstrated that corrosive bodily fluids weaken the implant surface and cause the detachment of the device from the bone. Moreover, Ti alloys with no coating may cause infection due to biofilm formation, and lead to implant failure due to stress corrosion.[12–15]

In people with a weakened immune system, chronic disease or addiction to tobacco products, the wound-healing processes are likely to be prolonged, which may lead to infection.[16] Researchers have suggested coating dental implants with a layer of antibiotics to reduce inflammation at the wound site.[17] In addition, a layer of agents such as amoxicillin, cephalothin and gentamycin can be applied to the implant surface to improve cellular responses.[18] Such an approach was adopted in an in vivo study, in which a biomaterial surface was coated with an antibiotic for a local release.[19]

The coating on implants plays a significant role in overcoming biocompatibility- and mechanical stress-related issues, and can withstand most biomechanical conditions. Nonetheless, improved performance has been achieved by coating metal implants with bioceramics, apatite-based coatings and ceramic/polymer composites.[20–26]

Bone quality plays a vital role in the customization of implants. Indeed, several studies have focused on bone quality to develop implants based on patient-specific mechanical responses.[27,28] Another important factor for determining the long-term success of dental implants is the load transferred from the implant to the surrounding bone.

Finite element analysis (FEA) is an important tool for predicting the behavior of the implant material and the bone. The finite element method (FEM) can be used to analyze several aspects of dental implant design, including the impact of implant thread design on stress distribution. Indeed, it can be used to identify parameters that have the greatest effect on stress distribution in the surrounding bone and the implant.[29–31]

A previous study demonstrated that the thickness and morphology of the hydroxyapatite (HAP) layer on dental implants could be optimized based on the stress values to improve implant stability.[32] Another study showed that an implant with a 150-micrometer-thick HAP layer minimized the stress concentrated in the surrounding bone.[33]

The main objective of this investigation was to study the influence of the coating thickness on patient-specific implants, and to optimize the coating thickness by using 3 different coating materials – HAP, monticellite (MTC) and titanium nitride (TiN).

Modeling and methods

Finite element modeling

A three-dimensional (3D) prototype of a section of the mandibular bone was designed. The prototype had a missing tooth, and the mandibular dimensions were derived from the literature.[33] The mandibular section consisted of 2 bones, including a core soft cancellous bone, which was 24.2 mm in height and had a width of 16.3 mm, and a 2-millimeter-thick hard cortical bone. A commonly used standard dental implant model (3.75 mm in diameter) (ISP-S 1338; Adin Dental Implants, Nagpur, India) and an 11.5-millimeter-long, aluminum oxide-blasted, acid-etched Ti alloy (Ti-6Al-4V) implant (Adin Dental Implants) were used for the analysis. All of the required dimensions of the implant and the abutment were extracted for modeling using a profile projector (Fig. 1). The 3D models were constructed with coatings that conformed to the exact shape of the implant. The coatings were applied at thicknesses of 50 μm, 100 μm, 150 μm, and 200 μm. Dental feldspathic porcelain was used for the crown and a cobalt-chromium (Co-Cr) alloy was used for the metal framework. The mandible was surrounded by a gum-like structure called gingiva and the implant was placed inside the mandible. The implants and the remaining components were modeled using the SOLIDWORKS® program, v. 2017

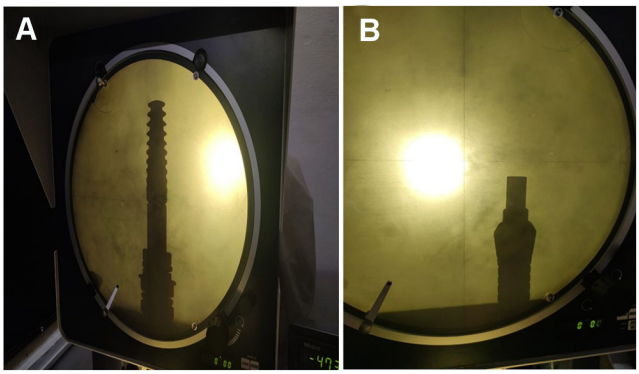

Fig. 1. Images of the dental implant (A) and of the abutment (B) in a profile projector

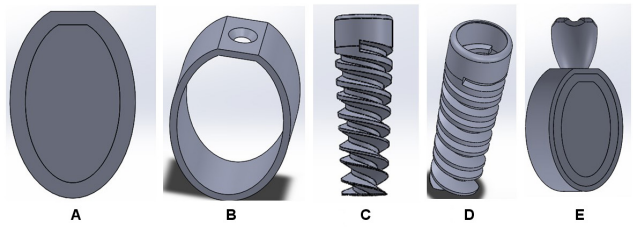

Fig. 2. Components used in the model

A – cancellous and cortical bones; B – gingiva; C – implant; D – coating layer; E – assembly of the dental implant.

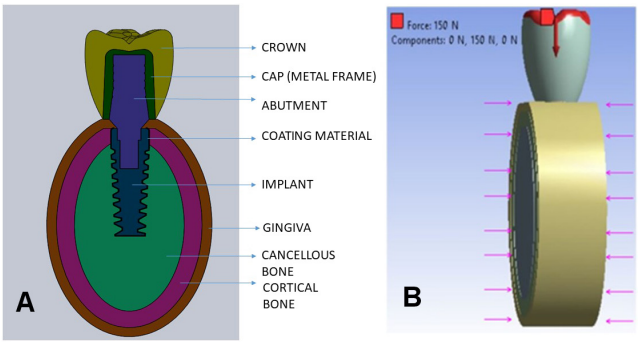

Fig. 3. Sectional view of the dental implant with the coating (A) and the dental implant with the coating at loading conditions (B): An axial compressive load of 150 N applied on the crown (the red arrow) and on both sides (from the right and from the left) of the mandibular bone section (the pink arrows)

(Dassault Systèmes, Vélizy-Villacoublay, France), and are shown in Fig. 2. The sectional view, along with loading, is shown in Fig. 3.

Finite element method

The models assembled in SOLIDWORKS were imported into the Ansys Workbench FEA tool, v. 18.2 (Ansys, Inc., Canonsburg, USA), and the file format was converted to a type that could be used for the analysis. The mesh size used throughout the modeling process was 0.2, which made it possible to obtain the convergence of the finite element model stress values; it was similar to that used by others.[35] The physical interactions in the contact areas of the implant–mandible section (cortical and cancellous), implant–abutment, abutment–metal framework, and metal framework–crown interfaces during loading were taken into account using the bonded surface-to-surface contact feature in the FEA tool. The mechanical properties of the materials used for the components in the assembly are shown in Table 1.

To mimic the behavior of the bone in the mandibular section, the cortical and cancellous bones were assigned 5 different material combinations by adjusting the Young's modulus value of healthy bone by ±20%.[28] The material properties of the varying combinations of the cortical and cancellous bones are shown in Table 2.[28]

The loading conditions for the implant was an axial compressive load of 150 N applied on the crown and on both sides (from the right and from the left) of the mandibular bone section, as shown in Fig. 3B. The induced stress (the equivalent von Mises stress), transformed from the applied compressive stress through the crown to the implant, as well as 3 different coating materials – HAP,[36] MTC[37] and TiN[38] – at thicknesses of 50 µm, 100 µm, 150 µm, and 200 µm, were evaluated by means of FEM.

It has been observed in previous studies that the mechanical behavior of the implant depends on bone quality. However, there is still a gap in the literature regarding the effect of coated dental implants on different bone conditions.[39] Therefore, in the present study, implants with a varied thickness of various coating materials were investigated under static loading conditions to ascertain the optimal properties of patient-specific coated implants.

Table 2. Properties of the materials used for the cortical and cancellous bones in 5 different combinations

Combination	Young's modulus [MPa]	
	cortical bone	cancellous bone
Combination 1	13,700 (0%)	1,370 (0%)
Combination 2	10,960 (–20%)	1,096 (–20%)
Combination 3	16,440 (+20%)	1,644 (+20%)
Combination 4	16,440 (+20%)	1,096 (–20%)
Combination 5	10,960 (–20%)	1,644 (+20%)

Table 1. Properties of the materials used in the model

Component of the model	Material	Young's modulus [GPa]	Poisson's ratio	Yield strength [MPa]	Reference No.
Crown	porcelain	14	0.35	29	34
Cap (metal frame)	Co-Cr alloy	220	0.30	720	36
Abutment	surgical graded stainless steel	187.5	0.33	190	39
Implant	Ti alloy (Ti-6Al-4V)	110	0.32	800	34
Gingiva	gingiva	0.0196	0.30	–	34
HAP coating	HAP	67	0.30	–	36
MTC coating	MTC	51	0.30	–	38
TiN coating	TiN	305	0.30	–	41

HAP – hydroxyapatite; MTC – monticellite; TiN – titanium nitride; Co – cobalt; Cr – chromium; Ti – titanium.

Results

The equivalent von Mises stress values were calculated for the 3 implant coating materials under the specified loading conditions. The coating materials – HAP, MTC and TiN – were assessed at thicknesses of 50 µm, 100 µm, 150 µm, and 200 µm. The static analysis was carried out with the use of the Ansys Workbench software, v. 18.2.

Stress induced in the cortical and cancellous bones (combination 1)

Figures 4 and 5A show the distribution of the induced stress and its effect, respectively, for bone combination 1. It was observed that the von Mises stress in the cortical bone was 83.77 MPa with the HAP coating at a thickness of 50 µm. Meanwhile, in the case of the HAP coating at a thickness of 200 µm, the generated stress was reduced by 87%. On the other hand, the effect of the von Mises stress in the cancellous bone with the HAP coating at a thickness of 200 µm was reduced by 90% (Fig. 4 and Fig. 5A).

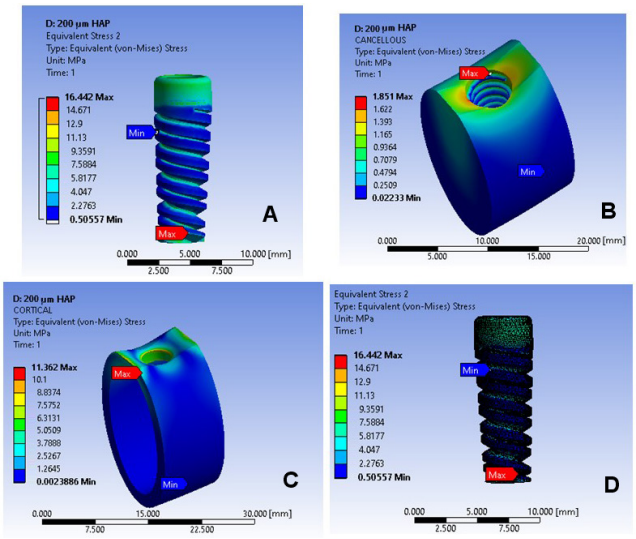

Fig. 4. Induced stress distribution for combination 1

A – stress at the coating layer (the HAP material); B – cancellous bone stress; C – cortical bone stress; D – implant stress; the arrows indicate the maximum and minimum stress at each component.

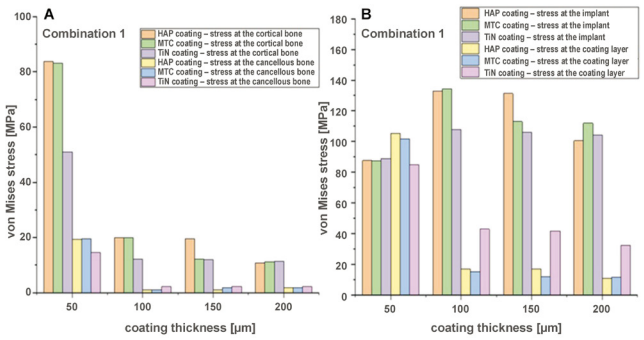

Fig. 5. Effect of the induced stress for combination 1

A – von Mises stress at the cortical and cancellous bones; B – von Mises stress at the implant and the coating layer.

Effect of stress on the implant and the coating layer (combination 1)

The stress value for the 100-micrometer HAP coating layer was 19.43 MPa, while it was reduced by nearly 90% for the 200-micrometer HAP coating layer with regard to 50 µm. The implant stress with the HAP coating was significantly different for combination 1 as compared to MTC and TiN (Fig. 5B).

Stress induced in the cortical and cancellous bones (combination 2)

Using combination 2, the maximum stress value in the cortical bone with the HAP coating at a thickness of 50 µm was over 50 MPa. In the cancellous bone, the maximum stress value with the 50-micrometer MTC coating was around 15 MPa. As the thickness of the coating increased, the stress in the cancellous bone decreased, with the 200-micrometer MTC coating reducing it by 78% (Fig. 6 and Fig. 7A).

Effect of stress on the implant and the coating layer (combination 2)

Figure 7B shows the stress values for combination 2 using coating thicknesses between 50 µm and 200 µm. The stress value for the MTC coating was reduced by 88% at a thickness of 200 µm. As the thickness of the coating layer increased, the implant stress increased as well, reaching as high as 113.81 MPa. The implant material used in this study (Ti-6Al-4V) has a yield strength ranging from 800 MPa to 950 MPa,[39] so the stress values were within the safe limits.

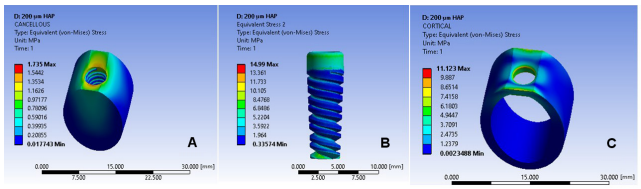

Fig. 6. Induced stress distribution for combination 2

A – cancellous bone stress; B – stress at the coating layer (the HAP material); C – cortical bone stress.

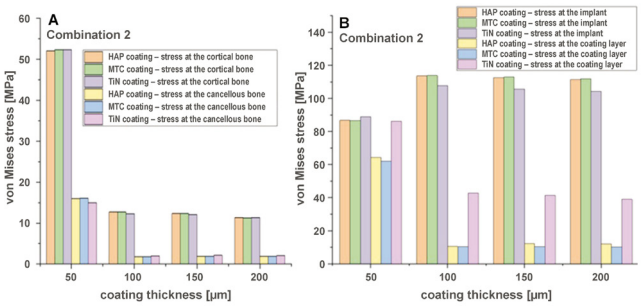

Fig. 7. Effect of the induced stress for combination 2

A – von Mises stress at the cortical and cancellous bones; B – von Mises stress at the implant and the coating layer.

Stress induced in the cortical and cancellous bones (combination 3)

For combination 3, the stress value was high at 50 µm and low at 200 µm (Fig. 8 and Fig. 9A). Using the 200-micrometer HAP coating, the stress was reduced by 87% in the cancellous bone.

Effect of stress on the implant and the coating layer (combination 3)

Figure 9B shows that as compared to the 50-micrometer coating, the stress was reduced by 81% for the 200-micrometer HAP coating, whereas there was an 83% reduction when using the 200-micrometer MTC coating. In addition, the stress at the implant was similar to that in combination 2, but was within the safe limits.

Stress induced in the cortical and cancellous bones (combination 4)

For combination 4, increasing the HAP coating thickness from 50 µm to 200 µm reduced the stress by 78% in the cortical bone. In the cancellous bone, the stress value decreased by 91% with the 200-micrometer thickness of the HAP coating as compared to the thickness of 50 µm (Fig. 10 and Fig. 11A).

Effect of stress on the implant and the coating layer (combination 4)

For the MTC coating material, the stress value was reduced by 84% for the 200-micrometer thickness as compared to 50 µm. Also, the stress at the implant was 87 MPa when using the 50-micrometer coating, and 119 MPa when using the 200-micrometer coating, which was within the safe limits (Fig. 11B).

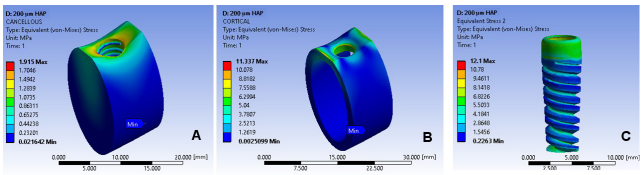

Fig. 8. Induced stress distribution for combination 3

A – cancellous bone stress; B – cortical bone stress; C – stress at the coating layer (the HAP material).

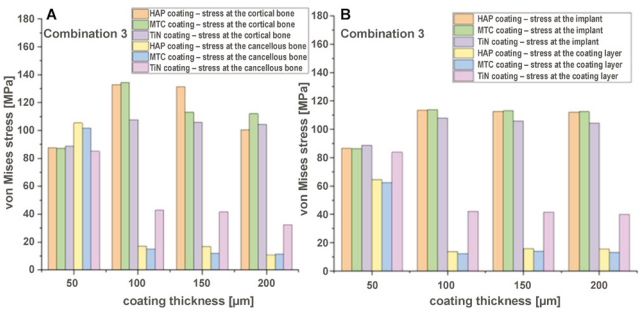

Fig. 9. Effect of the induced stress for combination 3

A – von Mises stress at the cortical and cancellous bones; B – von Mises stress at the implant and the coating layer.

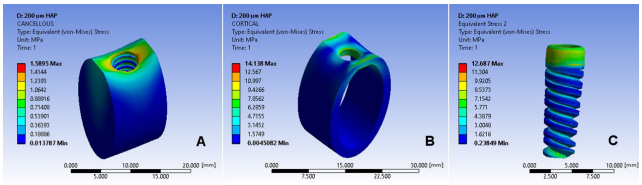

Fig. 10. Induced stress distribution for combination 4

A – cancellous bone stress; B – cortical bone stress; C – stress at the coating layer (the HAP material).

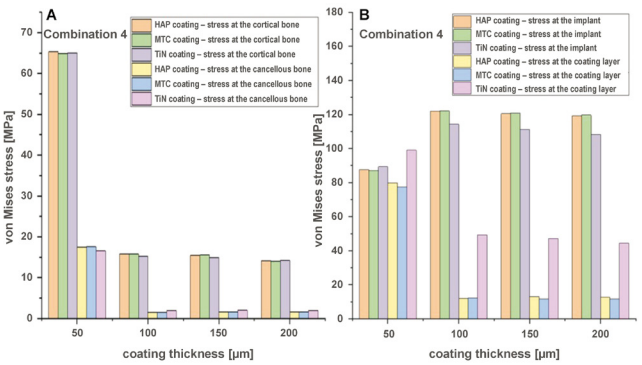

Fig. 11. Effect of the induced stress for combination 4

A – von Mises stress at the cortical and cancellous bones; B – von Mises stress at the implant and the coating layer.

Stress induced in the cortical and cancellous bones (combination 5)

For combination 5, the stress value in the cortical bone was reduced by 77% with the 200-micrometer HAP coating as compared to the 50-micrometer coating (Fig. 12 and Fig. 13A). A similar stress reduction in the cortical bone was found for the MTC coating material, where the stress was decreased by 77% by replacing the 50-micrometer coating with a 200-micrometer layer (Fig. 13A). In addition, the stress value in the cancellous bone was reduced by 83% when using the 200-micrometer HAP coating as compared to the 50-micrometer coating (Fig. 12 and Fig. 13A).

Effect of stress on the implant and the coating layer (combination 5)

Figure 13B shows that the HAP and MTC coating materials had a more significant role in decreasing the stress than TiN. Also, the stress was reduced by 72% by increasing the coating thickness from 50 µm to 200 µm. The maximum stress value observed for the HAP-coated implant was 107.83 MPa, although this was rather low as compared to combination 2.

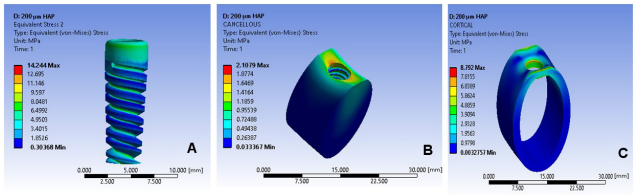

Fig. 12. Induced stress distribution for combination 5

A – stress at the coating layer (the HAP material); B – cancellous bone stress; C – cortical bone stress.

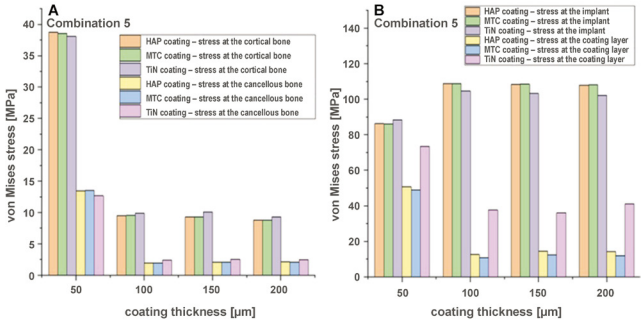

Fig. 13. Effect of the induced stress for combination 5

A – von Mises stress at the cortical and cancellous bones; B – von Mises stress at the implant and the coating layer.

Hypothesis testing

Statistical analysis was carried out on an uncoated implant model, which acted as a control. For combination 1, the mean stress value on coated implants was 87.29 MPa, while it was 89.33 MPa on uncoated implants. For combination 2, the mean stress value was 87.20 MPa on coated implants and 105.00 MPa on uncoated implants. For combination 3, the mean stress value on coated implants was 87.46 MPa and it was 76.00 MPa on uncoated implants. For combination 4, the mean stress value was 87.98 MPa on coated implants and 85.88 MPa on uncoated implants. Meanwhile, for combination 5, the mean stress value on coated implants was 86.84 MPa, and on uncoated implants, it was 84.31 MPa.

Here we accept the null hypothesis H_0 and reject the alternative hypothesis H_a. According to hypothesis testing:

H_0: $\mu = \mu_{H0} = 1$

The cases considered as H_a are as follows:

H_a: $\mu \neq \mu_{H0}$ The alternative hypothesis is that the population mean is not 1.

H_a: $\mu < \mu_{H0}$ The alternative hypothesis is that the population mean is less than 1.

H_a: $\mu > \mu_{H0}$ The alternative hypothesis is that the population mean is more than 1.

All the values obtained for the coatings ranged between 87 MPa and 88 MPa. Therefore, the difference is 1, which is a minimum value for variations. However, the values for uncoated implants ranged from 76 MPa to 105 MPa. Therefore, the difference in values is not equal to 1. Thus, the null hypothesis value is considered to be 1, and we accept H_0 and reject H_a. The decision chart is shown in Table 3.

Table 3. Decision-making process as to the hypotheses tested

Hypothesis	Decision	
	Accept	Reject
H_0 (true)	correct decision	error
H_0 (false)	error	correct decision

Discussion

The role of the coating on dental implants is to enhance osteoconduction, to fill gaps, and to create a bond between the bone and the implant. The selection of the coating material depends on various parameters, such as non-toxicity, corrosion resistance, fatigue strength, and durability. Hydroapatite bioceramic coatings have an additional advantage, as they reduce the healing time and have improved fixation strength.[39] The HAP coating thickness on dental implants ranges from 0 μm to 150 μm, and increasing the coating thickness reduces the induced stress and stress concentration.[33] Monticellite is a bioceramic that can form bone-like structures similar to the cortical bone,[40,41] while TiN coatings have a good load-carrying capacity.[42] For the above reasons, these 3 materials were selected as coatings in this modeling study, and were applied at thicknesses of 50 μm, 100 μm, 150 μm, and 200 μm, which is beyond those used previously (150 μm). In the present study, it was found that the optimum change in the induced stress was obtained between a coating thickness of 150 μm and 200 μm. Changing the thickness of any of the coating materials used from 150 μm to 200 μm had a significant impact, as the induced stress values were much lower as compared to coating thicknesses below 150 μm.

Bone quality is one of the parameters that affect stress distribution in the cortical and cancellous bones.[43] Furthermore, there is a high stress concentration in the area between the cortical and cancellous bones, as stated previously.[44] In the present work, a high stress concentration was observed at the neck area of the uncoated implant, which was reduced by coating the implant with a 200-micrometer layer. Figure 14 shows stress distribution for different bone

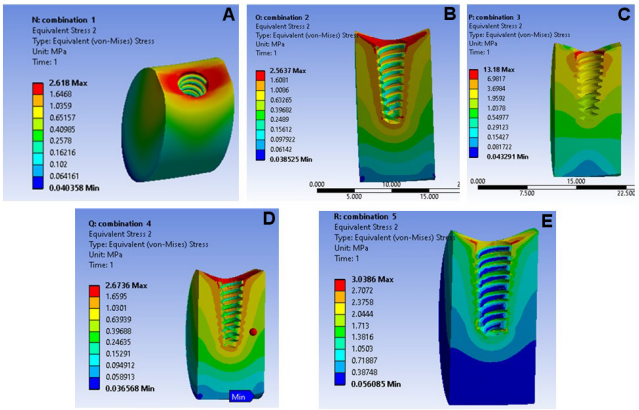

Fig. 14. Induced stress distribution in the cancellous bone for combination 1 (A), combination 2 (B), combination 3 (C), combination 4 (D), and combination 5 (E)

conditions, using uncoated implants. It was found that the 200-micrometer coating thickness reduced the stress values in the cancellous bone as compared to the uncoated implant by 34.3% (combination 1), 61.8% (combination 2), 149.0% (combination 3), 51.2% (combination 4), and 36.0% (combination 5).

Another important parameter is bone remodeling, which is a process that begins during the healing period.[45] It involves an increase in bone mass and high stress during bone formation (BF), while the stress is reduced during bone resorption (BR).[46] To ensure a successful BF process, more bone mass is needed. In this study, the microstrain values for coated implants ranged from 2,000 to 2,700 for all bone combinations. In contrast, the microstrain values for uncoated implants exceeded 3,700 for all bone combinations. Therefore, coated implants provide superior bone remodeling as compared to uncoated implants.

Limitations

The current study has several limitations, including the use of homogeneous and isotropic mechanical properties for the cortical and cancellous bones, and the simplified load conditions. Future studies should consider non-linear properties, dynamic loading, fatigue analysis, and contact analysis. Using more realistic material models would enable a better estimation of the mechanical environment at the bone–implant interface.

Conclusions

It can be concluded that the implants coated with HAP at a thickness of 200 μm were relatively superior in all 5 bone combinations. At the 200-micrometer coating thickness, both HAP and MTC gave better results than TiN. The most favorable coating thickness would be in the range of 150–200 μm, since in such conditions, the induced stress values significantly decreased for the cortical bone, the cancellous bone and the coating layer in this patient-specific study. Therefore, FEA could be used to design dental implants with patient-specific coatings.

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

Retrospective radiological analysis of cemento-osseous dysplasia

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

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Abstract

Background. Osseous dysplasia (OD) is a form of fibro-osseous lesion located in the jaws that may interfere with the adjacent anatomical structures.

Objectives. The aim of the present study was to evaluate the distribution of radiographic imaging features, the morphological characteristics and the lesion volume of OD with the use of cone-beam computed tomography (CBCT).

Material and methods. The study included radiologically diagnosed lesions followed up for at least 1 year. The prevalence and distribution of the OD types were defined in terms of age, sex, lesion location, teeth, relationship with the anatomical structures, and lesion volume.

Results. The mean age gradually increased from the periapical group to the florid group ($p = 0.018$). It was observed that the mandible was the most frequently affected bone (85.5%) ($p < 0.05$). The margins of the lesions were well defined, and had an irregular or circular shape. The buccal cortical bone was the most affected structure (84.5%), and the damage in the cortical bone increased with an increase in the lesion volume. With regard to teeth, the most frequent disorder was a discontinuous lamina dura (83.0%).

Conclusions. Osseous dysplasia lesions affect a wide range of different anatomical areas, and show different volume and morphometric characteristics.

Keywords: cone-beam computed tomography, bone diseases, florid cemento-osseous dysplasia

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Introduction

Most inflammatory periapical pathologies are a consequence of dental pulp necrosis. However, other types of lesions involve hard tissue formation and are not related to the condition of the pulp.[1]

Osseous dysplasia (OD), first described by Melrose et al.,[2] is a form of fibro-osseous lesion with unknown etiology and a periapical location. In such lesions, normal bone is replaced by fibrous tissue that contains amorphous vascularized calcifications.[3] Due to its close association with the periodontal ligament and shared histopathological features, some researchers have stated that the lesion originates in the periodontal ligament, and some have suggested that the condition may arise due to the defect in extra-ligamental bone remodeling caused by local factors associated with hormonal imbalance.[4,5]

The sub-classification of OD lesions is based on their location and extent rather than the histopathological process.[6] According to localization and the extent of jaw involvement, the lesions can be classified as periapical, focal or florid OD. In periapical OD, the lower anterior teeth are usually affected. Focal OD occurs in a single area of the posterior teeth. When the lesions involve 2 or more quadrants of the jaw, it is defined as florid OD.[7] Although the term 'familial gigantiform cementoma' was used in the past as a synonym for florid OD, most authors now restrict this term to an uncommon hereditary disorder that is significantly different from the conventional OD.

Florid OD is a gnathic bone disorder that ultimately leads to the formation of substantial sclerotic masses of disorganized mineralized material, and it is typically multi-focused on both the maxilla and the mandible.[8] Radiographically, it initially occurs as multiple radiolucencies in the periapical regions, but it later develops a mixed radiolucent and radiopaque model in the affected areas. Upon further maturation, the lesions become predominantly radiopaque, but often have a thin radiolucent margin.[9]

The radiographic appearance of all forms of OD can vary from hypodense, through mixed, to hyperdense areas due to lesion maturation over time and the accumulation of mineralized tissue. Initially, the radiolucent stage may be misdiagnosed as radicular cysts or periapical granulomas because of a periapical location. However, OD is usually associated with vital teeth, presents with no clinical complaints and requires no intervention. On the other hand, OD can be observed as mixed images, with residual cysts in edentulous areas, or as mixed/radiopaque images, with increasing density and calcification in later stages and lesion maturation over time. In this phase, it is vital to differentiate between osteomas, odontogenic tumors and idiopathic osteosclerosis.[10]

Although the lesions are usually well defined, their borders tend to be slightly irregular.[11] The lesions can sometimes be confused with other single or multiple lesions that are round or oblong, defined as bone islands or enostoses.[12] Idiopathic osteosclerosis is a localized radiopaque area of unknown etiology, also called a dense bone island or enostosis. It can be well circumscribed, usually round or oval, and sometimes irregularly shaped. It is distinguished from stage 3 OD based on not having a thin radiolucent edge.[13]

Panoramic and intraoral radiographs enable a limited examination of the adjacent anatomical structures. Cone-beam computed tomography (CBCT) allows a three-dimensional (3D) evaluation of anatomical structures with minimal distortion and high spatial resolution.[14] Thus, it has been quite beneficial for the detection of OD[15]; however, as the periapical form of OD has limited size and effects on the adjacent structures, CBCT may not be required to evaluate these lesions.

In previous studies, the lesion features and the effects of OD on anatomical structures were analyzed for the first time with the use of CBCT images to measure the lesion dimensions linearly in a Brazilian population.[3] The present study aimed to evaluate the radiomorphometric characteristics of cemento-osseous lesions in a Turkish population.

Material and methods

The ethics committee at the Faculty of Medicine of the Recep Tayyip Erdoğan University, Rize, Turkey, approved this research (reference number 2020/189), which complied with the Declaration of Helsinki.

The study included the data of patients who had reported to the Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Recep Tayyip Erdoğan University, Rize, Turkey, for various reasons and had been diagnosed with cemento-osseous lesions between 2017 and 2020. All patients signed an informed consent form. The study included radiologically diagnosed lesions followed up for at least 1 year. According to the radiographic follow-up, no patient required a biopsy. For stage 1 lesions, the vitality test results from the patient files were used to differentiate OD lesions from the endodontic ones. Thus, no histopathological data was obtained.

The lesions were divided into periapical, focal or florid OD, according to anatomical locations, clinical characteristics and radiographic features.[16] Lesions located only at the anterior teeth were classified as periapical, while those on a single posterior tooth were classified as focal, and those within 2 or more quadrants were classified as florid.

The exclusion criteria were as follows: a surgical intervention at the relevant areas; a history or the presence of orthodontic treatment; infected OD lesions; and CBCT data that did not include all the OD lesion areas.

All CBCT images were obtained using a Planmeca ProMax 3D Classic unit (Planmeca, Helsinki, Finland) with the following parameters: 90 kVp; 4–10 mA; and 200 μm

voxel size. The linear measurements were taken and the lesion characteristics were assessed using the Planmeca Romexis software, v. 4.6.2.R. The 3D reconstruction and the volume measurements were conducted using InVesalius, v. 3.1.0 (Centro de Tecnologia da Informação Renato Archer, Campinas, Brazil), and free open-source software (Fig. 1). The CBCT images were assessed in relation to the multiplanar reconstruction and the cross-sectional images, based on a study by De Oliveira Kato et al.[3]

Two oral and maxillofacial radiologists with over 10 and 6 years of experience, respectively, assessed the images independently. A random selection of 20% of the CBCT scans included in the study was reassessed after 2 weeks to evaluate intra-observer reliability. The kappa coefficients were 0.83 and 0.84 for observer 1 and observer 2, respectively, which indicates excellent intra-observer reliability. Inter-observer reliability demonstrated excellent agreement, with a kappa coefficient of 0.87.[17]

The details of the patients' age and sex, the associated lesions, the lesion location (right, left, anterior, and posterior), and the total number of locations were recorded. Lesion staging was based on the presence or absence of radiopaque calcifications in the lesion area. If there was none, the lesion was classified as stage 1, if a small calcified area that did not fill all of the lesion was present, it was classified as stage 2, and if the calcified area filled all of the cavity, with or without a slim radiolucent peripheral band, it was classified as stage 3. If the lesions were associated with the teeth, the tooth numbers were recorded. The periphery of the lesion was described as either well-defined (corticated, sclerotic, non-corticated, and partially corticated) or ill-defined (perforating, diffuse and invasive). The lesion shape was recorded as circular, oval or irregular. Hypodense capsules were assessed as present, absent or partially present. Involvement with the adjacent structures, such as the cortical bone (lingual and buccal), the mandibular canal, the mental foramen, the nasopalatinal canal, the incisive foramen, the maxillary sinus, the nasal fossa, and the anterior mandibular canal, was assessed and recorded. The effects on the cortical bone were assessed as follows: intact; thinning; expansion and thinning; thinning and perforation; expansion, thinning and perforation. The internal calcified parts of the lesion and the total lesion volume were also assessed.

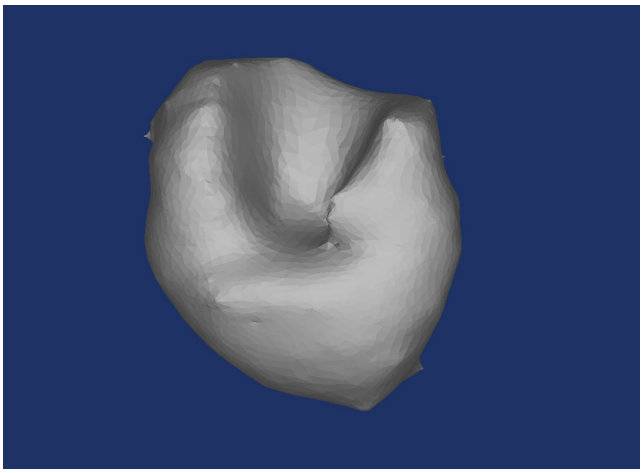

Fig. 1. Three-dimensional (3D) reconstruction of a cemento-osseous lesion

The greatest linear dimensions of the mesiodistal and buccolingual positions were measured on the axial plane, while the superoinferior dimension was measured on the cross-sectional images.

All the teeth associated with the OD lesion areas were recorded and assessed for the loss of lamina dura or periodontal ligament space, root resorption, tooth displacement, radiopacity adherent to the root, and endodontic treatment.

Statistical analysis

The IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA), was used to analyze the relationships between the different types of OD and the demographic and radiological data.

The distribution of the OD lesion types was determined using descriptive statistics and percentages. Additionally, differences in the width and depth of the lesions, according to the types and regions, were examined with the one-way analysis of variance (ANOVA) and the *t* test ($p < 0.05$). If a difference was noted, the Bonferroni test was used for multiple comparisons.

The study sample size was determined by conducting a power analysis, using G*Power (3.1.1).[18] The required sample size to reach a power of 0.90 with a type-one error (α) of 0.05 and a low effect size of 0.40 was calculated as 84.

Results

A total of 84 OD lesion areas were detected in 46 patients, including 5 males and 41 females. Female patients were more affected than male patients for all types of OD ($p < 0.05$).

The most frequent OD type was florid ($n = 17$; 37%), followed by focal ($n = 16$; 35%) and periapical ($n = 13$; 28%). Although the mean age was similar for all types of OD, it gradually increased from the periapical group to the florid group, and a statistically significant difference in the average age was observed between the periapical and florid groups ($p = 0.018$). In the periapical group, the mean age was 36.23 ±8.97 years (min: 24 years, max: 47 years), in the focal group, the mean age was 40.31 ±13.40 years (min: 21 years, max: 58 years), and in the florid group, the mean age was 47.53 ±8.46 years (min: 34 years, max: 68 years).

Irrespective of the OD type, the mandible (85.5%) was more involved than the maxilla (14.5%). The relationship between the total volume of the OD lesion areas and the cortical bone involvement is shown in Table 1.

Figure 2 shows the teeth associated with all groups. Taking into account all groups, the most frequently affected teeth were observed in the anterior region (66.0%).

When evaluating the groups separately, the buccal cortical bone (n = 15) and, in rare cases, the anterior mandibular canal (n = 4) were mostly affected by OD in the periapical group (Table 2). The affected anterior teeth often showed a discontinuous lamina dura, and an enlarged and non-uniformly visible periodontal ligament space. Hyperdense lesion areas adherent to tooth roots were observed in 2 cases (7.7%). Root resorption was not detected in any case, and tooth replacement was found only in 1 case (3.8%) (Table 3).

In the focal group, OD mostly affected the buccal (n = 13) and lingual (n = 9) cortical bone, followed by the mandibular canal (n = 6). The association with the mental foramen was detected in 2 cases and a maxillary sinus

Table 1. Changes in the cortical bone depending on the total volume of the osseous dysplasia (OD) lesion areas (Kruskal–Wallis test)

Effect on the cortical bone	*n*	Total volume of the OD lesion areas [mm^3]				*p*-value
		M ±*SD*	*Me*	min	max	
Intact	8	492.18 ±488.59	283.53	61.96	1,265.19	>0.05
Thinning	39	772.25 ±787.11	506.21	46.02	2,653.00	
Expansion and thinning	19	643.83 ±967.56	255.41	81.54	2,922.25	
Thinning and perforation	4	601.35 ±642.68	601.35	146.90	1,055.79	
Expansion, thinning and perforation	14	1,088.31 ±1,086.39	516.44	146.60	2,732.67	

M – mean; *SD* – standard deviation; *Me* – median; min – minimum; max – maximum.

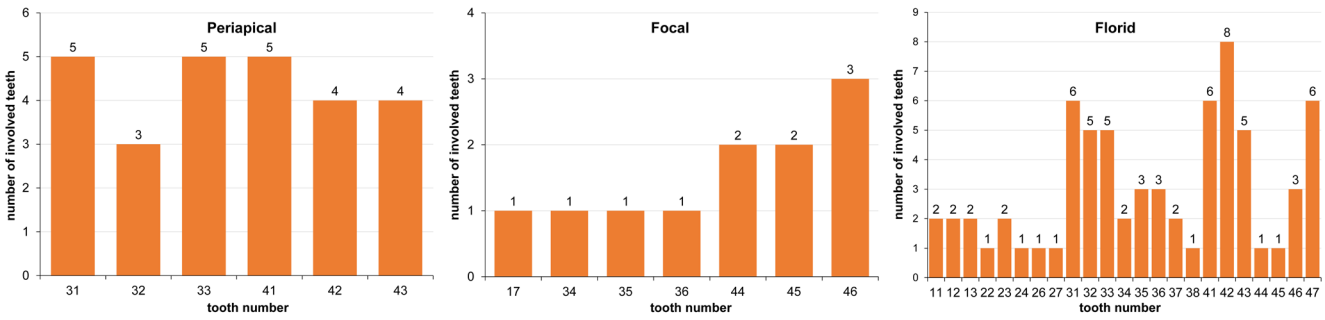

Fig. 2. Relationships between all types of osseous dysplasia (OD) lesions and the tooth numbers

Table 2. Distribution of the anatomical structures affected by periapical, focal and florid osseous dysplasia (OD)

Type of OD lesion	Anatomical structure	Mesiodistal dimension [mm]				Buccolingual/palatal dimension [mm]				Superoinferior dimension [mm]			
		n	*Me*	min	max	*n*	*Me*	min	max	*n*	*Me*	min	max
Periapical (n = 16)	cortical bone – lingual	13	7.40	3.79	18.84	13	6.40	4.02	8.25	13	7.60	2.80	11.21
	cortical bone – buccal	15	6.93	3.20	18.84	15	6.21	3.20	8.25	15	6.40	2.80	11.21
	anterior mandibular canal	4	9.38	6.93	18.84	4	6.73	6.21	6.83	4	7.60	6.40	10.22
Focal (n = 16)	cortical bone – lingual	9	8.02	5.00	15.01	9	7.06	4.57	9.65	9	7.40	5.40	10.00
	cortical bone – buccal	13	8.02	5.00	15.01	13	7.06	4.43	11.61	13	7.10	5.00	10.00
	mandibular canal	6	8.86	5.56	15.01	6	7.48	4.57	9.65	6	7.17	5.40	10.00
	mental foramen	2	6.79	5.56	8.02	2	4.84	4.57	5.11	2	5.86	5.40	6.32
	maxillary sinus	1	14.24	14.24	14.24	1	11.61	11.61	11.61	1	9.40	9.40	9.40
Florid (n = 52)	cortical bone – lingual	35	10.41	3.00	34.60	35	7.60	3.20	15.20	35	8.61	4.20	16.00
	cortical bone – buccal	43	8.60	3.20	34.60	43	7.21	2.40	15.20	43	8.20	2.83	16.00
	mandibular canal	13	10.41	4.00	19.40	13	8.21	3.62	14.04	13	12.00	6.60	13.60
	incisive foramen	2	8.10	5.80	10.40	2	5.54	3.80	7.28	2	7.34	5.00	9.67
	maxillary sinus	1	12.11	12.11	12.11	1	8.32	8.32	8.32	1	5.00	5.00	5.00
	nasal fossa	2	13.96	13.80	14.12	2	11.80	8.40	15.20	2	11.31	10.42	12.20
	anterior mandibular canal	1	5.66	5.66	5.66	1	6.40	6.40	6.40	1	4.80	4.80	4.80

relationship was detected in 1 case (Table 2). In the lesion areas, a well-defined periphery (n = 16; 100%), a partially corticated border (n = 7; 43.8%), a circular shape (n = 7; 43.8%), mixed density (n = 12; 75.0%), and a hypodense rim (n = 10; 62.5%) were detected (Table 4). The most frequently affected teeth were observed in the posterior region, especially on the right side (72.7%). While a discontinuous lamina dura and a change in periodontal ligament space were detected in 8 teeth (72.7%) in the lesion areas, no cases of root resorption or tooth displacement were found. In 2 cases, radiopaque masses adhered to the root (Table 3).

Table 3. Distribution of tooth alterations and the affected periodontal structures with regard to periapical, focal and florid osseous dysplasia (OD)

Variables	Periapical OD (n = 26)	Focal OD (n = 11)	Florid OD (n = 69)	Total (n = 106)
Lamina dura	24 (92.3)	5 (45.5)	59 (85.5)	88 (83.0)
Periodontal ligament space	21 (80.8)	3 (27.3)	47 (68.1)	71 (67.0)
Root resorption	0 (0)	0 (0)	1 (1.4)	1 (0.9)
Tooth displacement	1 (3.8)	0 (0)	5 (7.2)	6 (5.7)
Radiopacity adherent to the root	2 (7.7)	2 (18.1)	23 (33.3)	27 (25.5)
Endodontic treatment	0 (0)	0 (0)	2 (2.9)	2 (1.9)

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

The buccal (n = 43) and lingual cortical bone (n = 35) were mostly affected by lesions in the florid group. Moreover, displacement or perforation involved the mandibular canal in 13 cases, and the incisive foramen and the nasal fossa in 2 cases. In addition, the floor of the maxillary sinus (n = 1) and the anterior mandibular canal (n = 1) were related to displacement (Table 2). Most of the lesion areas demonstrated a well-defined periphery (n = 46; 88.5%), a partially corticated border (n = 20; 43.5%), an irregular shape (n = 22; 42.3%), mixed density (n = 28, 53.8%), and a hypodense rim (n = 39; 75.0%) (Table 4). The majority of lesions were found in 2 different areas (n = 8), with 5 observed in 3 different areas, 1 observed in 4, 1 observed in 5, and 2 extended to 6 different areas. The most frequently affected teeth were observed in the anterior of the mandible (50.7%), and 27.5% were on the right side. Most of the teeth affected by OD had a discontinuous lamina dura, and an enlarged and non-uniformly visible periodontal ligament space in the lesion area. Twenty-three teeth (33.3%) had hyperdense lesions adherent to tooth roots, while tooth displacement associated with OD was uncommon (Table 3).

Regarding age-related changes in radiographic features, radiolucent density was noted at the early stage of the lesion in the middle-age group (37.6 ±7.0 years), while in the 41.5 ±9.5 years age group, a mixed lesion stage was noticeable. In the group above the middle age (50.2 ±10.5 years), radiopaque masses with calcification were observed. In all types of OD, the volume of the OD lesion area was related to age, but the difference was not statistically significant (p = 0.056)

Table 4. Radiographic features of the lesion areas of periapical, focal and florid osseous dysplasia (OD)

Radiographic features		Groups			Total (N = 84)
		periapical (n = 16)	focal (n = 16)	florid (n = 52)	
Periphery	well-defined	15 (93.8)	16 (100)	46 (88.5)	77 (91.7)
	ill-defined	1 (6.3)	0 (0)	6 (11.5)	7 (8.3)
Well-defined	corticated	5 (33.3)	3 (18.8)	13 (28.3)	21 (27.3)
	sclerotic	0 (0)	2 (12.5)	4 (8.7)	6 (7.8)
	non-corticated	3 (20.0)	4 (25.0)	9 (19.6)	16 (20.8)
	partially corticated	7 (46.7)	7 (43.8)	20 (43.5)	34 (44.2)
Ill-defined	perforating	0 (0)	0 (0)	0 (0)	0 (0)
	diffuse	1 (100)	0 (0)	5 (83.3)	6 (85.7)
	invasive	0 (0)	0 (0)	1 (16.7)	1 (14.3)
Shape	circular	8 (50.0)	7 (43.8)	15 (28.8)	30 (35.7)
	oval	5 (31.3)	3 (18.8)	15 (28.8)	23 (27.4)
	irregular	3 (18.8)	6 (37.5)	22 (42.3)	31 (36.9)
Internal density	hypodense	5 (31.3)	0 (0)	6 (11.5)	11 (13.1)
	hyperdense	2 (12.5)	4 (25.0)	18 (34.6)	24 (28.6)
	mixed	9 (56.3)	12 (75.0)	28 (53.8)	49 (58.3)
Hypodense rim	present	14 (87.5)	10 (62.5)	39 (75.0)	63 (75.0)
	absent	1 (6.3)	1 (6.3)	3 (5.8)	5 (6.0)
	partially present	1 (6.3)	5 (31.3)	10 (19.2)	16 (19.0)

In all types of OD, the lesion area in each of the 3 dimensions showed no significant differences ($p > 0.05$). In the case of lesions located in the anterior and posterior regions, the mesiodistal distance was not significantly different. However, the posterior lesions showed a larger width for the buccolingual distance (7.40 ±2.74 mm) as compared to the anterior lesions (5.86 ±2.04 mm) ($p < 0.01$).

Discussion

Osseous dysplasia lesions may lead to endodontic misdiagnosis.[19] The disease is typically asymptomatic, and is usually detected when radiographs are recorded for other purposes. Correct diagnosis can pose problems, but it is crucial for proper management.[20] In most instances of OD, the distinctive clinical and radiographic patterns allow a strong presumptive diagnosis without the necessity of performing a biopsy.[21]

Periapical OD primarily involves the periapical region of the anterior mandible. The early stage of the lesion appears as a limited radiolucent area covering the apical region of the tooth. Solitary lesions may occur, but multiple foci are encountered more often.[22]

Focal OD can occur in any area of the jaw, but the posterior side of the mandible is the most affected region. Radiographically, the lesion varies from completely radiolucent to densely radiopaque with a thin radiolucent peripheral rim. However, a mixed radiolucent and radiopaque pattern is most commonly found.[23]

Florid OD presents with multifocal involvement and is not limited to the anterior mandible. Although many cases demonstrate multifocal lesions only in the posterior portions of the jaw, there are a lot of patients who also exhibit anterior mandible involvement.[6] The lesions demonstrate a marked tendency for bilateral and often quite symmetrical involvement, and it is not unusual to encounter extensive lesions in all 4 posterior quadrants.[24] Initially, the lesions are predominantly radiolucent. However, they become mixed over time and predominantly radiopaque with only a thin radiolucent peripheral rim. Occasionally, the lesion can become almost completely radiopaque and blend with the adjacent normal-appearing bone.[25]

Extensive knowledge about the characteristics of OD, correct diagnosis and the interpretation of CBCT images are crucial to avoid incorrect treatment. Therefore, this study described the demographic and general radiographic features of OD in a Turkish population, using the volume measurement and 3D technology, and compared them with the data in the existing literature. To our knowledge, this is the first study to describe the size of OD by volume with the use of CBCT.

Based on the findings of the present retrospective study, the tendency for females to exhibit OD lesions appears to be higher as compared to males. This finding is compatible with other reports in the existing literature.[15,26] In previous studies, the lesions were more common in black women in the 5th and 6th decades, and it was suggested that genetic and/or hormonal factors may be causative.[26]

Osseous dysplasia is usually detected by routine diagnostic radiographs. However, advanced imaging methods are needed to evaluate some differences in the focal and florid OD variants, and to reveal the relationships between the lesions and anatomical structures.[27] In previous reports, almost 90% of OD lesions affected the mandible and they usually occurred adjacent to the lower teeth, above the lower alveolar canal.[25,28] Also, the focal and florid types affect the posterior area of the mandible, while the periapical group exhibits a predilection for the anterior mandible.[29] The findings of the current study support previous results.[26,30] In the present study, the mandible was more affected than the maxilla. Surprisingly, in addition to the periapical group, the most affected area in the florid group was the anterior region and it mostly involved 2 quadrants of the mandible.

There is no consensus among studies on the frequency of subgroups. In a retrospective study conducted in North and South America, the most common type reported was periapical OD.[30] Meanwhile, an international multicenter study from Africa reported that florid OD was the most common form.[31] In the present study, the most common types identified among 46 diagnosed cases of OD were focal and florid. This result may be related to the fact that florid OD is more symptomatic than other types. However, the groups also exhibited changes with respect to age. The florid group appeared at an older age (47.53 ±8.46 years) and the periapical group appeared at a younger age (36.23 ±8.97 years). In the middle-aged group, uniformly radiolucent density and the mixed stage were predominant, while in the group above the middle age, homogeneous radiopaque masses with calcification were most commonly observed. This general result is in accordance with previous radiographic data.[32] Such increased radiopacity indicates a greater degree of avascular mineralized tissue formation.

Relationships with anatomic structures in the current study were similar to the previously reported ones.[33] In this study, OD lesions affected both the buccal and lingual cortical bone ($n = 49$), the mandibular canal ($n = 19$), the anterior mandibular canal ($n = 5$), and the mental foramen ($n = 2$). In addition, the incisive foramen ($n = 2$), the maxillary sinus ($n = 2$) and the nasal fossa ($n = 2$) were involved. Generally, the lesions were characterized by well-defined partially corticated, hypodense images of an irregular shape with mixed density. The presence of hypodense capsules can facilitate OD diagnosis. In the present study, they were mostly detected in the periapical and florid groups. However, in 37.5% of lesions in the focal group, hypodense capsules were partially or completely absent. Therefore, care should be taken when diagnosing focal OD.

Panoramic radiography is an easily accessible and routinely used imaging method in clinical dentistry, but it is a two-dimensional (2D) modality with disadvantages such as superposition and magnification. Furthermore, it is insufficient to determine the radiological characteristics of OD lesions in a millimeter-scale examination of anatomical structures, e.g., the periodontal ligament space, lamina dura and the cortical bone. Cone-beam computed tomography is a frequently preferred imaging method in the diagnosis of maxillofacial pathologies due to its 3D evaluation feature, a lower radiation dose, and sub-millimeter image resolution as compared to computed tomography (CT).[34] In the present study, the lesion volumes were determined using 3D images, and cortical bone changes were compared with volume measurements for the first time.

The anatomical structure most affected was the cortical bone, especially the buccal cortex. The thinning of the cortical bone was the most common type of damage observed. However, when the lesion volume increased, the bone damage changed from intact to perforation, and when the total calcified volume was evaluated, it was significantly lower in the periapical group. When age-related changes were assessed, calcification was detected earlier in the periapical group than in the other groups. The amount of calcified tissue in the lesion increased with time and reached stage 3. Differences in the amount of calcified tissue between the groups may result from age differences.

With regard to the 2D measurements taken in previous studies, the lesions show greater growth in the mesiodistal direction than in other planes.[3,28] Additionally, in this study, mesiodistal directionality and greater growth in the superoinferior direction were also observed, which may indicate that the lesions do not display tumoral growth and expand to the cancellous bone.

The discontinuity of lamina dura was observed in 83.0% of cases, and an irregular periodontal ligament space was found in 67.0%. In total, only 5.7% of cases involved tooth displacement. Similar results were obtained in another 3D study, where the authors emphasized that the persistence of pulp vitality, along with the absence of tooth mobility, supported the outcome of the harmonic disorder between periodontal structures and OD lesions.[3] It also supported the theory of the origin of the lesion from the periodontal ligament tissue.[3] On the contrary, in a previous 2D imaging study, 77.6% of lamina dura was intact and periodontal ligament space was normal in 92.1% of cases.[30] However, this may be related to the limitations of 2D imaging.

In this study, only 1 patient in the florid group exhibited root resorption. Similarly, few studies have reported root resorption associated with OD,[15,30] whereas De Oliveira Kato et al. reported a significant number of cases demonstrating root resorption.[3] Root resorption is considered an indicator of damage severity.[35] Endodontic, orthodontic and periodontal factors that may cause a root change must be eliminated.

Limitations

The limitations of this study are related to its retrospective cross-sectional design and the evaluation of a limited number of cases. Further information on the radiological features of OD lesions is required by expanding the study with more cases.

Conclusions

In conclusion, OD was more prevalent in female patients, and the anterior mandible was the most affected bone in all types of OD. Osseous dysplasia lesions mostly affected anatomical structures by thinning the buccal cortical bone. Also, in 3D evaluation, the volume of the lesions increased, and the damage to the surrounding cortical bone also increased. The 3D assessment of frequently detected OD lesions and their clinical morphological features is extremely important for monitoring lesion development, distinguishing the lesions from other pathologies and observing their relationships with the surrounding anatomical structures.

Ethics approval and consent to participate

The research was approved by the ethics committee at the Faculty of Medicine of the Recep Tayyip Erdoğan University, Rize, Turkey, approved this research (reference number 2020/189). All patients 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

Frequency of symptoms and the associated factors of eating disorders in a group of dental students in Medellín, Colombia

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Conflict of interest
None declared

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Abstract

Background. Eating disorders (EDs) are considered a public health problem. Scientific research has focused on teenagers due to their higher prevalence in this population. However, other groups, such as university students, may be exposed to suffering from EDs due to their academic, social and personal characteristics. Identifying the magnitude of EDs and the associated characteristics may impact the generation of prevention strategies.

Objectives. The aim of the present study was to establish the prevalence of the risk of EDs (anorexia nervosa (AN) and bulimia nervosa (BN)) and the associated factors in dental students at the University of Antioquia, Medellín, Colombia..

Material and methods. A cross-sectional study was conducted on 278 students (76 males, 202 females) with the use of a Google-Forms survey. The validated Spanish version of the Eating Attitudes Test-26 (EAT-26) and the Sick, Control, One Stone, Fat, Food (SCOFF) questionnaire were used. Other recorded variables included sociodemographic data, coronavirus disease 2019 (COVID-19)-related factors, health, and social support (the Duke-11 profile). The bivariate analysis of the risk of EDs was conducted according to different variables (95% confidence interval (*CI*)), followed by the logistic regression models adjusting for different variables (adjusted prevalence ratio (aPR) and 95% *CI*).

Results. According to EAT-26, the risk of EDs was 27.6% (18.8–38.6) for males and 28.7% (22.9–35.3) for females. However, differences between males and females were higher when the SCOFF questionnaire was applied (males: 6.6% (2.8–14.5); females: 22.3% (17.1–28.5); $p < 0.01$). According to the SCOFF instrument, after adjusting for sociodemographic and health variables, women were more likely to report the symptoms of EDs (aPR: 2.20; 95% *CI*: 1.06–4.57). Women receiving information from social networks were more likely to report the symptoms of EDs (aPR: 1.85; 95% *CI*: 1.19–2.88). Multivariate models showed that women reporting poor self-rated health and some symptoms during the mandatory confinement caused by the COVID-19 pandemic were more likely to report the symptoms of EDs.

Conclusions. A higher risk of EDs was found in dental students. Healthcare, psychological and/or psychiatric interviews, and educational/early prevention strategies are required.

Keywords: health surveys, dental students, feeding and eating disorders, anorexia nervosa, bulimia nervosa

Introduction

Eating disorders (EDs) represent a physical and mental challenge, and are considered a global public health problem.[1] According to "Diagnostic and Statistical Manual of Mental Disorders (DSM-5™)", feeding and eating disorders are characterized by a persistent disturbance of eating or eating-related behavior that results in the altered consumption or absorption of food, which significantly impairs physical health or psychosocial functioning.[2] The EDs proposed for this classification are anorexia nervosa (AN), bulimia nervosa (BN), binge eating disorder (BED), other specified feeding or eating disorder (OSFED), pica, rumination disorder, avoidant/restrictive food intake disorder (ARFID), and unspecified feeding or eating disorder (UFED); other proposed criteria include muscle dysmorphia and orthorexia nervosa (ON).[2] In general terms, the most frequent EDs in adolescent and adult populations are AN, BN and BED, whereas the remaining disorders are characteristic of childhood.[2]

According to a systematic review of studies on EDs in the 2000–2018 period, despite differences in the tools used for evaluating and classifying EDs, the prevalence of EDs varies from 2.2% to 19.4% for females, and from 0.7% to 13.8% for males.[1] Although studies on adolescent populations (specifically females) are most common, recent epidemiological studies have focused on other groups, such as university students, and middle-aged and older persons.[3–5]

University students constitute a potential high-risk population for EDs,[4,5] especially students in the healthcare specialties.[6] They are vulnerable to high stress levels and burnout, and often suffer from mental health problems.[7] An association between EDs and depressive symptoms has been shown.[8,9] In addition, the possible influence of changes in the nutritional status, eating patterns and diet, as well as the role social media and networks play in self-esteem, the corporal image and body satisfaction/dissatisfaction, are recognized as strongly related to the prevalence of EDs.[10–13] It cannot be overlooked that the coronavirus disease 2019 (COVID-19) pandemic significantly affected university students' mental health, and their depression and stress levels, and resulted in the presence of EDs and other related risk behaviors.[14]

In Colombia, research has been conducted on several populations, including studies on female gym-goers,[15,16] the analysis of EDs in males to identify clinical subtypes and relate them to psychological symptoms,[17] and epidemiological studies on undergraduate students from public and private universities.[18,19] Nevertheless, specific studies on dental students are scarce. Determining the magnitude of EDs and the associated characteristics may lead to timely diagnosis and strategies for prevention in specific populations.[20]

Accordingly, the present study aimed to establish the prevalence of the risk of EDs and the associated factors, focusing on AN and BN in dental students at the University of Antioquia, Medellín, Colombia.

Methods

Design, data collection and setting

This cross-sectional study used an anonymous survey administered online to a sample of dental students (undergraduate/postgraduate) at the Faculty of Dentistry of the University of Antioquia, Medellín, Colombia. The final sample of respondents was 278 (27% males and 73% females). The study fieldwork was conducted between March and April 2021. The questionnaire was designed using the Google Forms platform (available upon request), and distributed through digital media, including Facebook groups, WhatsApp messages, e-mails, and institutional invitations. Participation was voluntary. The questionnaire gathered information about sociodemographic data, and included questions about the COVID-19 pandemic, social networks, health conditions, and self-reported EDs. We did not receive any incomplete questionnaires. To avoid duplicate questionnaires and to guarantee that the study population filled out the survey, the Google Form requested a valid e-mail address.

Variables

Dependent variables

The primary analysis used 2 instruments. First, the validated Spanish version of the Eating Attitudes Test-26 (EAT-26) was applied for the detection of the ED risk.[21,22] It is a questionnaire with 26 Likert-type questions (88.9% sensitivity and 97.7% specificity). Each question has 6 response options (never, rarely, sometimes, often, very often, or always); the first 3 were rated 0, the fourth 1, the fifth 2, and the sixth 3. The total score of EAT-26 is the sum of the 26 items. They score 3, 2, 1, 0, 0, 0 in the positive direction, assigning 3 to responses that most closely approximate a symptomatic direction (always = 3). Only item 25 is scored in the opposite way, scoring 0, 0, 0, 1, 2, 3 (never = 3). The cut-off point used to determine the ED risk according to EAT-26 was ≥11, based on one study of diagnostic validity and usefulness conducted in Medellín.[21] A second complementary approach, the Sick, Control, One Stone, Fat, Food (SCOFF) questionnaire, was used as a screening test for EDs. It is a questionnaire with 5 yes/no questions. A SCOFF score with at least 2 positive answers indicates an ED.[23,24]

Independent variables

The independent variables were as follows: sociodemographic data, including age, the type of academic training, the socioeconomic status, and the number of people at home; the level of COVID-19 knowledge; social support; and the perception of social networks. Social support was assessed using the Duke-11 Functional Social Support Questionnaire among the participants. This self-administered instrument is composed of 11 statements, using a five-point Likert-type response scale (1 = much less than I want, 2 = less than I want, 3 = neither much nor little, 4 = almost as much as I want, and 5 = as much as I want). A cut-off point was validated at the 32-point level, suggesting that a score lower than this refers to low social support, while a score equal to or higher than this indicates an adequate level.[25]

Health conditions

The following health conditions were taken into account:

– self-rated health (good/poor);
– the body mass index (BMI), defined as a person's weight in kilograms divided by the square of their height in meters (kg/m^2); according to the norms established by the World Health Organization (WHO),[26] BMI ≤ 18.49 kg/m^2 was regarded as underweight, BMI between 18.50 kg/m^2 and 24.99 kg/m^2 as normal weight, BMI between 25.00 kg/m^2 and 29.99 kg/m^2 as overweight, and BMI ≥ 30.00 kg/m^2 as obesity (in the present study, self-reported BMI was used, i.e., the measure was based on the responses of the surveyed people about their weight and height);
– the WHO Five Well-Being Index (WHO-5), applied to measure the subjective well-being of the participants. This instrument comprises 5 statements: 'I have felt cheerful and in a good mood'; 'I have felt calm and relaxed'; 'I have felt active and energetic'; 'I have woken up refreshed and rested'; and ''My daily life has been full of things that interest me'. They are rated on a Likert scale from 0 to 5, where 0 = never, 1 = occasionally, 2 = less than half the time, 3 = more than half the time, 4 = most of the time, and 5 = all the time. Subsequently, the scores were added to obtain a total value that was multiplied by 4 to give a figure ranging from 0 to 100, where the higher the score, the greater the well-being[27];
– the level of concern regarding COVID-19;
– sleep quality during mandatory social isolation (COVID-19);
– the presence of certain symptoms during mandatory social isolation (COVID-19): headache; insomnia; muscular pain; appetite loss; libido loss; fatigue; irritability/moodiness; lack of concentration; anxiety; and eye strain;
– consumption habits during mandatory social isolation (COVID-19): coffee; alcohol; smoking; and psychoactive substances.

Statistical analysis

A pilot test was carried out with a sample of 14 participants to improve intelligibility, and to assess the completion time and internal consistency. Cronbach's alpha was 0.89 for EAT-26 and 0.63 for SCOFF. Test-retest reliability was calculated for 9 participants, obtaining values of 0.99 for EAT-26 and 0.87 for SCOFF.

All analyses were conducted separately for males and females. Descriptive analysis was carried out for dependent and independent variables (qualitative and quantitative). The prevalence of EDs with 95% confidence intervals (95% *CI*s) were calculated for both instruments (EAT-26 and SCOFF) according to independent variables. The χ^2 tests were used to establish statistically significant differences among the study variables. Furthermore, multivariate logistic regression analyses were conducted. Initially, an association between the presence of EDs and sex was determined. Secondly, specific associations between the selected independent variables and the presence of ED were calculated. We estimated the odds ratios (ORs) with 95% *CI*s, and later converted them to adjusted prevalence ratios (aPRs), using the mathematical formula proposed by Miettinen (Equation 1)[28]:

$$aRP = \frac{OR}{1 + p_1 \times (OR - 1)} \qquad 95\%\ CI = OR^{\left(1 \pm \frac{1.96}{Z}\right)} \qquad (1),$$

where p_1 represents the prevalence of the disease in the reference group, and Z is the regression coefficient divided by its standard error.

The conversion was carried out based on the assumption that OR tends to overestimate the strength of association with a prevalence higher than 10%. In both cases (OR-aPR), the statistical significance and the magnitude of associations was assessed. Statistical analyses employed IBM SPSS Statistics for Windows, v. 25.0 (IBM Corp., Armonk, USA).

Ethical considerations

Adhering to international standards for online surveys, all respondents completed an informed consent statement on the first page of the questionnaire, and the participants could reject or approve involvement in the study. Confidentiality was guaranteed throughout the research process in accordance with the Declaration of Helsinki. The Ethics Committee at the Faculty of Dentistry of the University of Antioquia approved the study (Act 03-2021, Concept 76). This manuscript followed the standards for observational studies, as established by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.[29]

Results

Table 1 shows the sociodemographic characteristics of the study population and the prevalence of EDs. According to EAT-26, the ED risk was 27.6% (18.8–38.6) for males and 28.7% (22.9–35.3) for females. However, differences between males and females were higher when the SCOFF questionnaire was applied (males: 6.6% (2.8–14.5); females: 22.3% (17.1–28.5); $p < 0.01$). Both instruments reported a higher risk in female undergraduate students under 24 years of age, with a high socioeconomic status, living with 5 or more people at home, and with low perceived social support. However, statistically significant differences were found for the variable academic training when the SCOFF scores were considered. Similarly, the score that gages the influence of social networks on these disorders was higher in those at risk for EDs ($p < 0.05$) and those getting their information about EDs from social networks ($p < 0.01$). For men, applying EAT-26 revealed a striking level of knowledge about COVID-19 among those at risk for EDs ($p < 0.01$). The SCOFF questionnaire showed a higher ED risk among male undergraduate students over 25 years of age, with a medium socioeconomic status, living with 4 or fewer people at home, and with low social support (no statistically significant differences were found). The prevalence of ED risk was higher in those who get information about these disorders from social networks ($p < 0.05$).

Table 2 shows health conditions and substance consumption during the mandatory social isolation caused by the COVID-19 pandemic and the prevalence of ED risk. For females, both instruments revealed a higher prevalence of ED risk among those reporting poor health ($p < 0.01$ for EAT-26 and $p < 0.05$ for SCOFF), those who

Table 1. Prevalence of eating disorders (EDs) according to sociodemographic data and the perception of social networks in the study population ($N = 278$)

Variables			Males			Females		
			sample *n* (%)	EAT-26 % (95% *CI*)	SCOFF % (95% *CI*)	sample *n* (%)	EAT-26 % (95% *CI*)	SCOFF % (95% *CI*)
Socio-demographic data	age [years]	≤24	51 (67.1)	31.4 (20.3–45.0)	5.9 (2.0–15.9)	140 (69.3)	32.1 (25.0–40.3)	24.3 (17.9–32.0)
		≥25	25 (32.9)	20.0 (8.9–39.1)	8.0 (2.2–25.0)	62 (30.7)	21.0 (12.7–32.6)	17.7 (10.2–29.0)
	type of academic training	undergraduate	65 (85.5)	27.7 (18.3–39.6)	7.7 (3.3–16.8)	182 (90.1)	29.7 (23.5–36.7)	24.2 (18.2–30.9)*
		postgraduate	11 (14.5)	27.3 (9.8–56.6)	0.0	20 (9.9)	20.0 (8.1–41.6)	5.0 (0.9–23.6)*
	socioeconomic status	low	24 (31.6)	41.7 (24.5–61.2)	4.2 (0.7–20.2)	82 (40.6)	25.6 (17.4–36.0)	17.1 (10.5–26.6)
		medium	50 (65.8)	20.0 (11.2–33.0)	8.0 (3.2–18.8)	105 (52.0)	30.5 (22.5–39.8)	24.8 (17.5–33.8)
		high	2 (2.6)	50.0 (9.5–90.6)	0.0	15 (7.4)	33.3 (15.2–58.3)	33.3 (15.2–58.3)
	number of people at home	≤4	62 (81.6)	27.4 (17.9–39.6)	8.1 (3.5–17.5)	144 (71.3)	28.5 (21.7–36.3)	22.2 (16.2–29.7)
		≥5	14 (18.4)	28.6 (11.7–54.7)	0.0	58 (28.7)	29.3 (19.2–42.0)	22.4 (13.6–34.7)
Level of COVID-19 knowledge *Me* (*IQR*)			7.0 (7.0–8.0)	7.0 (6.5–7.0)**	6.0 (5.0–7.0)	7.0 (6.0–8.0)	7.0 (6.0–8.0)	7.0 (6.0–8.0)
Social support (Duke-11)		normal	69 (90.8)	26.1 (17.2–37.5)	5.8 (2.3–14.0)	177 (87.6)	26.6 (20.6–33.5)	20.9 (15.6–27.5)
		low	7 (9.2)	42.9 (15.8–75.0)	14.3 (2.6–51.3)	25 (12.4)	44.0 (26.7–62.9)	32.0 (17.2–51.6)
Perception of social networks	influence of social networks on eating habits *Me* (*IQR*)		5.0 (2.0–7.5)	6.0 (2.0–8.0)	4.0 (3.0–7.0)	6.0 (3.0–8.0)	7.5 (4.8–9.0)*	8.0 (5.0–9.5)*
	information about EDs from social networks	no	52 (68.4)	25.0 (15.2–38.2)	1.9 (0.3–10.1)*	90 (44.6)	21.1 (14.0–30.6)*	11.2 (7.0–20.6)**
		yes	24 (31.6)	33.3 (18.0–53.3)	16.7 (6.7–35.9)*	112 (55.4)	34.8 (26.6–44.1)*	30.4 (22.6–39.4)**
	relevance and usefulness of information from social networks regarding eating habits and EDs *Me* (*IQR*)		6.0 (5.0–8.0)	6.0 (5.0–7.5)	7.0 (6.0–7.5)	6.0 (4.0–8.0)	6.0 (5.0–8.0)	6.0 (4.0–8.0)
Total			76 (27.3)	27.6 (18.8–38.6)	6.6 (2.8–14.5)**	202 (72.7)	28.7 (22.9–35.3)	22.3 (17.1–28.5)**

EAT-26 – Eating Attitudes Test-26; SCOFF – the Sick, Control, One Stone, Fat, Food questionnaire; *CI* – confidence interval; COVID-19 – coronavirus disease 2019; *Me* – median; *IQR* – interquartile range; Duke-11 – the Duke-11 Functional Social Support Questionnaire; * $p < 0.05$; ** $p < 0.01$. In the case of quantitative variables, the *Me* (*IQR*) values are shown for the study participants reporting the ED risk according to EAT-26 and SCOFF.

Table 2. Prevalence of eating disorders (EDs) according to health conditions and consumption habits in the study population (*N* = 278)

Variables			Males			Females		
			sample *n* (%)	EAT-26 % (95% *CI*)	SCOFF % (95% *CI*)	sample *n* (%)	EAT-26 % (95% *CI*)	SCOFF % (95% *CI*)
Health conditions	self-rated health	good	68 (89.5)	26.5 (17.5–38.0)	7.4 (3.2–16.1)	170 (84.2)	24.7 (18.8–31.7)**	19.4 (14.2–26.0)*
		poor	8 (10.5)	37.5 (13.7–69.4)	0.0	32 (15.8)	50.0 (33.6–66.4)**	37.5 (22.9–54.8)*
	BMI	underweight	5 (6.6)	60.0 (23.1–88.2)	0.0	13 (6.4)	23.1 (8.2–50.3)	15.4 (4.3–42.2)
		normal	54 (71.1)	25.9 (16.1–38.9)	5.6 (1.9–15.1)	145 (71.8)	27.6 (21.0–35.4)	20.0 (14.3–27.3)
		overweight/ obesity	17 (22.4)	23.5 (9.6–47.3)	11.8 (3.3–34.3)	44 (21.8)	34.1 (21.9–48.9)	31.8 (20.0–46.6)
	WHO-5 *Me* (*IQR*)		64.0 (56.0–76.0)	64.0 (46.0–72.0)	68.0 (42.0–78.0)	56.0 (44.0–68.0)	48.0 (36.0–56.0)**	48.0 (38.0–56.0)**
	level of concern regarding COVID-19 *Me* (*IQR*)		6.5 (5.0–8.0)	7.0 (5.0–8.0)	8.0 (6.0–8.0)	7.0 (5.0–8.0)	7.0 (5.0–8.0)	7.0 (5.0–8.0)
	sleep quality during mandatory social isolation (COVID-19) *Me* (*IQR*)		7.0 (5.0–8.0)	7.0 (5.5–8.0)	7.0 (5.5–7.5)	7.0 (4.0–8.0)	5.5 (3.0–7.0)**	6.0 (4.0–7.0)**
	presence of symptoms during mandatory social isolation (COVID-19)	headache	25 (32.9)	28.0 (14.3–47.6)	4.0 (0.7–19.5)	109 (54.0)	33.9 (25.7–43.3)	29.4 (21.6–38.5)**
		insomnia	40 (52.6)	22.5 (12.3–37.5)	7.5 (2.6–19.9)	103 (51.0)	35.9 (27.3–45.5)*	28.2 (20.4–37.5)*
		muscular pain	12 (15.8)	33.3 (13.8–60.9)	0.0	44 (21.8)	43.2 (29.7–57.8)*	29.6 (18.2–44.2)
		appetite loss	13 (17.1)	46.1 (23.2–70.9)	0.0	38 (18.8)	47.4 (32.5–62.7)**	31.6 (19.1–47.5)
		libido loss	6 (7.9)	66.7 (30.0–90.3)*	0.0	25 (12.4)	48.0 (30.0–66.5)*	28.0 (14.3–47.6)
		fatigue	16 (21.1)	31.3 (14.2–55.6)	6.3 (1.1–28.3)	50 (24.8)	44.0 (31.2–57.7)**	34.0 (22.4–47.9)*
		irritability/ moodiness	29 (38.2)	31.3 (17.3–49.2)	3.5 (0.6–17.2)	92 (45.5)	38.0 (28.8–48.3)**	33.7 (24.9–43.8)***
		lack of concentration	34 (44.7)	29.4 (16.8–46.2)	2.9 (0.5–14.9)	109 (54.0)	31.2 (23.3–40.4)	27.5 (20.0–36.6)
		anxiety	22 (28.9)	45.5 (26.9–65.3)*	9.1 (2.5–27.8)	116 (57.4)	41.4 (32.8–50.5)***	33.6 (25.7–42.6)***
		eye strain	29 (38.2)	27.6 (18.8–38.6)	3.5 (0.6–17.2)	97 (48.0)	35.1 (26.3–45.0)	26.8 (19.0–36.4)
Consumption habits during mandatory social isolation (COVID-19)	coffee	decreased	11 (21.2)	10.0 (1.8–40.4)	0.0	26 (18.3)	19.2 (8.5–37.9)**	15.4 (6.2–33.5)*
		equal	28 (53.8)	25.0 (12.7– 40.4)	7.1 (2.0–22.7)	72 (50.7)	22.2 (14.2–33.1)**	19.4 (12.0–30.0)*
		increased	13 (25.0)	38.4 (17.7–64.5)	7.7 (1.4–33.3)	44 (31.0)	47.7 (33.8–62.1)**	38.6 (25.7–53.4)*
	alcohol	decreased	34 (54.0)	26.5 (14.6–43.1)	8.8 (3.1–23.0)	58 (48.7)	25.9 (16.4–38.4)*	22.4 (13.6–34.7)
		equal	21 (33.3)	19.1 (7.7–40.0)	4.8 (0.9–22.7)	39 (32.8)	38.5 (24.9–54.1)*	25.6 (14.6–41.1)
		increased	8 (12.7)	37.5 (13.7–69.4)	0.0	22 (18.5)	59.1 (38.7–76.7)*	40.9 (23.3–61.3)
	smoking	decreased	4 (33.3)	0.0	0.0	10 (37.0)	20.0 (5.7–51.0)	10.0 (1.8–40.4)*
		equal	2 (16.7)	0.0	0.0	15 (55.6)	26.7 (10.9–52.0)	26.7 (10.9–52.0)*
		increased	6 (50.0)	66.7 (30.0–90.3)	16.7 (3.0–56.4)	2 (7.4)	100.0 (34.2–100.0)	100.0 (34.2–100.0)*
	psychoactive substances	decreased	6 (46.2)	16.7 (3.0–56.4)	16.7 (3.0–56.4)	11 (37.9)	18.2 (5.1–47.7)	0.0*
		equal	6 (46.2)	50.0 (18.8–81.2)	0.0	15 (51.7)	20.0 (7.1–45.2)	33.3 (15.2–58.3)*
		increased	1 (7.7)	100.0 (20.7–100.0)	0.0	3 (10.3)	66.7 (20.8–93.9)	66.7 (20.8–93.9)*

BMI – body mass index; WHO-5 – the WHO (World Health Organization) Five Well-Being Index; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. In the case of quantitative variables, the *Me* (*IQR*) values are shown for the study participants reporting the ED risk according to EAT-26 and SCOFF. In the case of qualitative variables, the frequency values for the ED risk are shown for the study participants reporting symptoms during mandatory social isolation (COVID-19).

are overweight/obese (with no statistically significant difference), those with low general well-being scores ($p < 0.01$), and those with poor sleep quality during mandatory social isolation ($p < 0.01$). Similarly, a higher prevalence of ED risk was found in females reporting specific symptoms during isolation, and statistically significant differences were observed, depending on the symptom and the instrument used for reporting the ED risk (Table 2). For males, EAT-26 found a higher prevalence of ED risk among those reporting poor health, being underweight, and experiencing the loss of libido ($p < 0.05$) or anxiety ($p < 0.05$).

Regarding substance consumption during the social isolation caused by the pandemic (Table 2), the SCOFF questionnaire showed a higher prevalence of ED risk in females, with statistically significant differences ($p < 0.05$) for those with increased consumption of coffee and psychoactive substances, and increased smoking. The EAT-26 indicator reported a higher prevalence of ED risk in females with increased consumption of coffee and alcohol ($p < 0.01$ and $p < 0.05$, respectively). When analyzing men, no statistically significant differences were observed. However, there was a higher prevalence of ED risk reflected by EAT-26 in those who reported increased consumption of the aforementioned substances.

A multivariate association analysis compared the presence of ED symptoms with regard to sex, as shown in Fig. 1. According to SCOFF, females were more likely to report the symptoms of EDs, and this association was maintained after adjusting for sociodemographic and health variables (aPR: 2.20; 95% *CI*: 1.06–4.57).

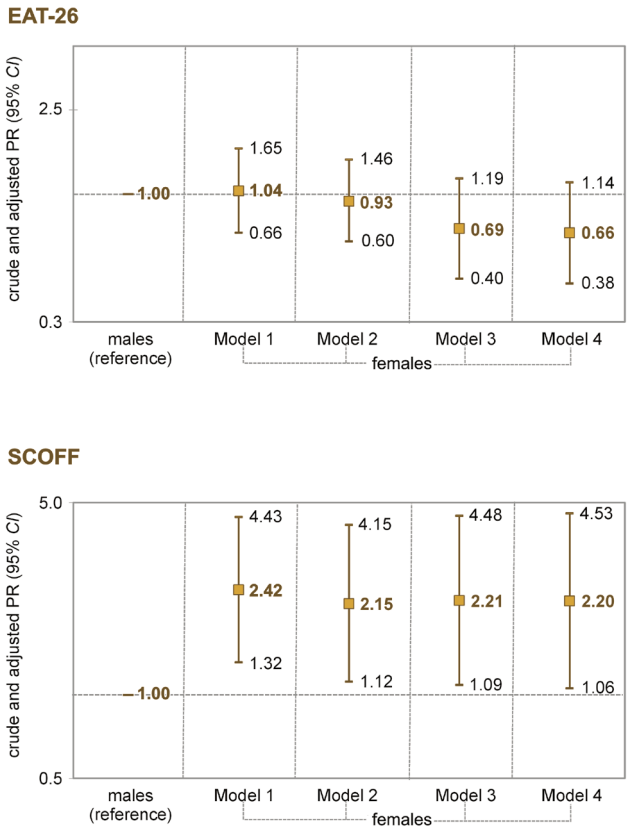

Fig. 1. Association between eating disorders (EDs) and sex according to the EAT-26 and SCOFF instruments (*N* = 278)

PR – prevalence ratio. Model 1: crude (unadjusted) PR; Model 2: adjusted PR (aPR) for sociodemographic data and the perception of social networks; Model 3: aPR for health variables; Model 4: aPR for sociodemographic and health variables.

Table 3. Association between eating disorders (EDs) and sociodemographic data and the perception of social networks in the study population (*N* = 278)

Variables			EAT-26 % (95% *CI*)				SCOFF % (95% *CI*)			
			males		females		males		females	
			Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Socio-demographic data	age [years]	≤24	1.45 (0.72–2.94)	1.81 (0.72–4.55)	1.43 (0.92–2.21)	1.34 (0.79–2.25)	0.73 (0.13–4.23)	0.43 (0.06–3.22)	1.33 (0.77–2.30)	0.87 (0.45–1.68)
		≥25	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	type of academic training	undergraduate	1.01 (0.38–2.69)	1.22 (0.23–6.56)	1.40 (0.67–2.93)	1.30 (0.45–3.76)	–	–	2.72 (0.88–8.47)	3.28 (0.94–11.45)
		postgraduate	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	socioeconomic status	low–medium	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		high	1.46 (0.50–4.27)	1.55 (0.53–4.49)	1.16 (0.57–2.35)	0.86 (0.33–2.27)	–	–	1.44 (0.39–5.31)	1.78 (0.86–3.65)
	number of people at home	≤4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		≥5	1.04 (0.41–2.65)	1.22 (0.49–3.06)	1.03 (0.65–1.62)	1.03 (0.62–1.70)	–	–	1.01 (0.60–1.68)	0.85 (0.47–1.57)
Social support (Duke-11)		normal	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		low	1.43 (0.67–3.07)	1.41 (0.64–3.13)	1.43 (0.93–2.13)	1.44 (0.95–2.19)	2.18 (0.35–13.62)	2.26 (0.27–18.80)	1.42 (0.81–2.50)	1.54 (0.85–2.78)
Perception of social networks	information about EDs from social networks	no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		yes	1.29 (0.67–2.48)	1.31 (0.68–2.56)	1.48 (1.03–2.14)	1.40 (0.96–2.06)	4.02 (1.04–15.51)	4.20 (1.09–16.21)	1.90 (1.25–2.89)	1.85 (1.19–2.88)

Model 1: crude (unadjusted) PR; Model 2: aPR for the variables considered.

Table 3 illustrates the multivariate analysis of the association between the symptoms of EDs and sociodemographic variables. According to SCOFF, females receiving ED information from social networks were more likely to report the symptoms of EDs (aPR: 1.85; 95% *CI*: 1.19–2.88). In the remaining categories, no statistically significant differences were found in the adjusted model.

Finally, Table 4 shows the multivariate analysis of the association between the symptoms of EDs and health variables. According to EAT-26, after adjusting for variables, females with poor self-rated health (aPR = 1.44; 95% *CI*: 1.04–2.00), muscular pain (aPR = 1.50; 95% *CI*: 1.07–2.11), appetite loss (aPR = 1.48; 95% *CI*: 1.01–2.47), fatigue (aPR = 1.46; 95% *CI*: 1.05–2.03), irritability/moodiness (aPR = 1.49; 95% *CI*: 1.06–2.08), and anxiety (aPR = 1.87; 95% *CI*: 1.36–2.56) were more likely to report the symptoms of EDs. In the SCOFF questionnaire, after adjusting for variables, females reporting headache (aPR = 1.64; 95% *CI*: 1.03–2.60), irritability/moodiness (aPR = 1.86; 95% *CI*: 1.26–2.73) and anxiety (aPR = 2.16; 95% *CI*: 1.45–3.23) were more likely to report the symptoms of EDs.

Discussion

The main study findings showed that females suffer from ED symptoms more frequently than males, though other sociodemographic factors and health conditions had a differential influence on the risk of these disorders.

Table 4. Association between eating disorders (EDs) and health conditions in the study population (*N* = 278)

Variables			EAT-26 % (95% *CI*)				SCOFF % (95% *CI*)			
			males		females		males		females	
			Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Self-rated health		good	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		poor	1.33 (0.56–3.17)	1.26 (0.48–3.34)	1.51 (1.13–2.00)	1.44 (1.04–2.00)	–	–	1.60 (1.05–2.42)	1.43 (0.87–2.36)
BMI	underweight		1.44 (0.90–2.32)	–	0.82 (0.27–2.47)	0.79 (0.21–3.01)	–	–	0.76 (0.20–2.90)	0.95 (0.22–4.03)
	normal		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	overweight/obesity		0.91 (0.52–1.58)	0.83 (0.28–2.46)	1.21 (0.77–1.90)	1.25 (0.77–2.03)	1.97 (0.41–9.42)	1.59 (0.27–9.38)	1.46 (0.92–2.32)	1.65 (1.01–2.70)
Presence of symptoms during mandatory social isolation (COVID-19)	headache	no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		yes	1.02 (0.44–2.36)	0.91 (0.40–2.07)	1.40 (0.96–2.03)	1.32 (0.89–1.95)	0.50 (0.25–1.00)	0.36 (0.06–4.47)	1.76 (1.14–2.69)	1.64 (1.03–2.60)
	insomnia	no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		yes	0.64 (0.28–1.47)	0.49 (0.19–1.26)	1.50 (1.06–2.12)	1.36 (0.93–1.99)	1.34 (0.25–7.29)	1.12 (0.17–7.17)	1.57 (1.01–2.44)	1.37 (0.84–2.24)
	muscular pain	no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		yes	1.22 (0.53–2.81)	1.34 (0.55–3.25)	1.48 (1.07–2.04)	1.50 (1.07–2.11)	–	–	1.38 (0.85–2.25)	1.50 (0.89–2.54)
	appetite loss	no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		yes	1.53 (0.91–2.57)	1.43 (0.81–2.50)	1.50 (1.12–2.00)	1.48 (1.01–2.47)	–	–	1.45 (0.90–2.35)	1.17 (0.66–2.08)
	libido loss	no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		yes	1.39 (1.01–1.91)	1.40 (0.98–2.00)	1.48 (1.05–2.08)	1.43 (0.98–2.07)	–	–	1.27 (0.67–2.42)	1.14 (0.56–2.32)
	fatigue	no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		yes	1.16 (0.52–2.57)	1.31 (0.57–3.02)	1.51 (1.12–2.04)	1.46 (1.05–2.03)	0.93 (0.10–8.74)	0.73 (0.08–7.00)	1.59 (1.06–2.37)	1.54 (0.98–2.42)
	irritability/moodiness	no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		yes	1.19 (0.61–2.33)	1.07 (0.51–2.25)	1.55 (1.12–2.13)	1.49 (1.06–2.08)	0.39 (0.04–3.56)	0.32 (0.02–4.46)	1.90 (1.32–2.73)	1.86 (1.26–2.73)
	lack of concentration	no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		yes	1.11 (0.57–2.20)	1.18 (0.57–2.45)	1.19 (0.80–1.78)	1.04 (0.64–1.70)	0.30 (0.03–2.88)	0.33 (0.03–3.37)	1.56 (0.99–2.45)	1.51 (0.91–2.51)
	anxiety	no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		yes	1.61 (1.19–2.18)	1.18 (0.96–2.70)	1.91 (1.27–2.89)	1.87 (1.36–2.56)	1.60 (0.31–8.27)	0.69 (0.07–6.45)	2.17 (1.50–3.15)	2.16 (1.45–3.23)
	eye strain	no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		yes	1.00 (–)	0.78 (0.32–1.88)	1.41 (0.99–2.02)	1.34 (0.92–1.96)	0.39 (0.04–3.56)	0.44 (0.04–5.20)	1.41 (0.89–2.23)	1.36 (0.83–2.24)

Model 1: crude (unadjusted) PR; Model 2: aPR for the variables considered.

Furthermore, when the association analyses using multivariate models were conducted, some ED risk factors maintained their statistical significance, especially for females, including the influence of social networks, specific health conditions and the symptoms perceived during the mandatory confinement caused by the COVID-19 pandemic.

Sex as an analytic category played an important role when possible differences in the prevalence of ED risk were considered among the dental students participating in this study. However, the magnitude of these differences was higher (by 120% for females) when the SCOFF questionnaire scores were analyzed, and no statistically significant differences were found with EAT-26. An international systematic review shows that ED symptoms are more frequent in females,[1] although the prevalence observed for both sexes in our study was higher than that reported globally and for the Americas. Other studies conducted on university students show different findings. For instance, studies from Pakistan[4] and Malaysia[5] using the EAT-26 questionnaire showed no statistical differences between the sexes. A study from Tunisia[6] conducted on health occupation students (including dentistry), using the SCOFF questionnaire showed a higher risk prevalence in males (27.3% vs. 6.6%) and females (36.3% vs. 22.3%) as compared to the present Colombian study. Finally, a Colombian study using the SCOFF questionnaire in a private university (with minimal participation of dentistry students) found that females were more likely to report ED symptoms, including AN and BN (OR = 1.6; 95% *CI*: 1.2–2.1).[19]

Several aspects should be taken into account when analyzing the results of different studies that considered sex. First, there are differences in the precision and specificity of the instruments used to capture populations at risk for EDs, and this situation is reflected in the varying frequencies of these disorders.[1,3] According to a meta-analysis of the diagnostic accuracy of the SCOFF questionnaire, a relatively low sensitivity was found for the male population, and the efficacy of the test was marginally higher for females.[30] Second, analyzing the cultural patterns related to diet and food intake, and the aspects concerning corporal dissatisfaction is pertinent, as these factors occur differently between men and women.[31,32] For instance, according to a Mexican study, women are significantly more obsessed with thinness and experience body dissatisfaction, and men prefer to do physical exercise to control their weight.[33] Scientific evidence indicates that men perceive EDs to be more common and "acceptable" as compared to women.[34] Third, an association with psychological aspects, such as stress, depression and anxiety, should be considered in the analysis of EDs according to sex.[9] Finally, specific analyses of risk behaviors and EDs in men are lacking. One study conducted in Colombia, a case series of 21 men, presented 4 clinical subtypes: restrictive, impulsive, avoidant, and secondary to other psychopathology.[17]

Some differences in the prevalence of ED symptoms aligned with sociodemographic variables. Although no significant differences were observed across all cases, bivariate and multivariate analyses showed certain trends. In this sense, the interpretation of the findings may be difficult, since the studies conducted on university students did not deeply explore the association of ED symptoms with sociodemographic variables, as in our study in Colombia. For instance, one study conducted on Malaysian university students found a lower risk of EDs in older students (similar to Colombia).[5] On the other hand, in our study, the participants with low social support were more likely to report EDs. A qualitative analysis conducted on 22 females showed that social support contributed to ED recovery through an individual's sense of connection to self and others.[35] Further research is needed to explore socioeconomic, family and academic factors that influence EDs.

Regarding the participants' BMI, we found no conclusive data. For instance, underweight males reported the ED risk more frequently on the EAT-26 test, while overweight/obese females reflected the ED risk more frequently on both tests (EAT-26 and SCOFF). Nevertheless, in multivariate models, statistical significance was maintained for females (on the SCOFF questionnaire). It should be noted that the BMI calculated for this study was based on self-reported input (weight and height). Studies on university students from Malaysia[5] and Spain[31] reported that a higher BMI was related to ED risk factors. These students were worried about their bodies and felt fear of gaining fat, which could explain body shape dissatisfaction.[5,31] Also, the image of the ideal body may differ between the sexes.

Another element worth analyzing is that the study period in Colombia coincided with the COVID-19 pandemic. The fieldwork was carried out in the 1st quarter of 2021, and at that time, the measures restricting face-to-face activities at the university were in effect, which involved important adaptations in lifestyle and study habits for our research participants. Clinical patient care activities alternated with the educational activities mediated by virtual tools, and the vaccination of dental students was prioritized under the policies and strategies of the national government.[36] For that reason, the study findings may have been influenced by the pandemic. Other studies have shown that the COVID-19 lockdown increased the risk of EDs at the global level,[37] especially among university students.[14]

Complementing the above, our study found several associations between health indicators and ED symptoms. The study participants who experienced the ED risk were more likely to report symptoms during mandatory social isolation (COVID-19), including poor mental health, a decline in well-being and changes in consumption habits, although the magnitude and statistical significance of their influence depended on the indicator and the type of analysis (bi- or multivariate). These results are not

readily comparable, since not all studies used these health indicators to establish associations with EDs. However, the findings highlight the effects of the pandemic on the mental and physical health of the university population. For instance, studies on dental students in Saudi Arabia showed the detrimental effects of the COVID-19 lockdown on mental health, such as anxiety, stress and depression.[38,39]

The changes in consumption habits during COVID-19 isolation uncovered in our study indicate that the participants' smoking increased, as did their coffee, alcohol and psychoactive substance intake, and they reported higher frequencies of ED symptoms. In females, the differences were statistically significant. A Malaysian study did not find associations between current tobacco use and the ED risk,[5] while a study on health occupation students from Tunisia found a higher frequency of ED symptoms in individuals reporting regular alcohol use and a lower frequency in smokers (in both cases, the differences were not statistically significant).[6] Our results draw attention to the impact of the pandemic on the consumption of the aforementioned substances[40] and underscore their effects on the mental health of university students.[41]

Mass media, the Internet and social networks might exert influence on risk and disorder behaviors, as shown by previous studies.[11,12,15] Our study found that the participants receiving information about EDs from social networks were more likely to report symptoms (especially females when multivariate analyses were applied). In this sense, it is advisable to explore the impact of the Internet-based information on the ED risk, the possibility of following influencers that affect self-perception, and beauty ideals that may influence self-esteem and cause extreme food preoccupation.[11,12,15]

Strengths and limitations

The strengths of this study are that the research tools were previously validated by pilot testing, and the primary outcomes were evaluated by verified tests (EAT-26 and SCOFF), ensuring high sensitivity, specificity and consistency. Nevertheless, the study limitations must be considered when interpreting its findings. The cross-sectional nature of this research did not permit causal relationships to be analyzed. It seems important to consider the characteristics of Internet-based questionnaire studies. Despite the Google Form requesting an e-mail address to avoid duplicates (a large proportion of participants gave their institutional e-mails), some participants may have just accidentally found the questionnaire and filled it out. Similarly, the research group was not able to get information on why some members of the studied group (dental students in this case) did not take part in the study. The ED tests may only be used as a screening strategy for the possible detection of the population at risk and do not constitute a definitive diagnosis of these disorders. Thus, psychological/psychiatric interviews are required to identify specific EDs and establish treatment plans according to the patient's needs.

Notwithstanding the above limitations, this study contributes to the knowledge about EDs that university students may experience, especially with regard to sociodemographic factors, their health status and the effects of the COVID-19 pandemic. Further research could elucidate some specific aspects, such as EDs in males, and use more precise tests that would differentiate the types of EDs, with specific analyses of the body image and food intake. In addition, qualitative studies that would allow an approach to the social representations and onset of EDs through the experience of university students in their academic, social and personal contexts should be undertaken.

Conclusions

A higher frequency of ED risk was observed among dental students who participated in this study. In ED prevalence, differences were found according to sociodemographic factors, certain health conditions and the psychological effects of the pandemic, some associated with the influence of social networks. These findings represent useful input for establishing timely diagnosis strategies, with appropriate professionals to initiate educational activities for health promotion and early prevention and to generate epidemiological surveillance systems that would allow the monitoring of the incidence/prevalence of these disorders in the short, medium and long term.

Ethics approval and consent to participate

Adhering to international standards for online surveys, all respondents completed an informed consent statement on the first page of the questionnaire, and the participants could reject or approve involvement in the study. Confidentiality was guaranteed throughout the research process in accordance with the Declaration of Helsinki. The Ethics Committee at the Faculty of Dentistry of the University of Antioquia approved the study (Act 03-2021, Concept 76). This manuscript followed the standards for observational studies, as established by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

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

Force decay and elongation of orthodontic elastomeric chains exposed to different beverages common in the diet: An in vitro study

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Abstract

Background. Elastomeric chains promote controlled movements and are widely used in orthodontics.

Objectives. The aim of the study was to evaluate the force decay and elongation of orthodontic chains exposed to low-pH saliva (pH = 4) and different beverages common in the diet.

Material and methods. Force decay and elongation were determined in vitro at 6 time intervals over 21 days for 2 commercial elastomeric chains – Ormco (group A) and Borgatta (group B). The samples were immersed in artificial saliva (AS) at pH 4, Coca-Cola®, coffee, or beer for 15 min every day, or in AS (the control group). For the remaining time, the chains were placed into AS at 37°C. In addition, microscopic qualitative changes were recorded by means of scanning electron microscopy (SEM).

Results. The group B chains showed higher force decay and elongation at the end of the follow-up as compared to the group A chains. Exposure to beer had a greater impact on the force decay and elongation of the chains as compared to other liquids, but it was not statistically significant. The group A chains showed a more irregular surface than the group B chains, in particular, those exposed to coffee.

Conclusions. Elastomeric chains suffer force decay and elongation as a function of time, mainly in the first 24 h. At the end of the follow-up, the group A chains exhibited less force decay in comparison with the group B chains. The qualitative observations showed that the chains in group A had a more irregular surface than the chains in group B.

Keywords: surface properties, orthodontics, material testing, fixed appliances

Introduction

In orthodontics, dental biomechanics uses force systems to produce controlled dental movements. Elastomeric chains promote these controlled movements, and are widely used for the closure of dental spaces, canine distalization, the correction of dental rotations and midline discrepancies, and the orthodontic traction of impacted teeth; they serve to hold the arches in brackets and act as a substitute for metal ligatures.[1] Although the precise composition of elastomeric chains is an industry secret, polyurethanes are known to be their primary component, and orthodontists have used the chains widely since the 1960s.[2]

Elastomeric chains are easy to handle, low-cost and do not require patient cooperation. However, the main disadvantages of elastomers are force decay and deformation. After a time, the initial magnitude of the force applied by the chain is reduced, and therefore, the movement of the teeth can decrease or stop. On the other hand, the deformation of the material can be elastic or plastic. When the applied force exceeds the elastic limit of the material, the chain shows a permanent alteration, i.e., chain elongation.[3]

The properties of the chains may be affected by factors such as the low pH of saliva. Beverages and foods can induce important changes in the oral environment in terms of pH, buffer capacity, titratable acidity, viscosity, and concentrations of calcium, phosphate and fluoride.[4] One study provided participants with a carbonated beverage with a pH of 2.45, and showed that 10 min after consuming the drink, the pH on the surfaces of the teeth dropped to 4.[5] Likewise, the present study used artificial saliva at pH 4 to mimic the conditions in the mouth after the consumption of an acidic beverage.

Many common drinks have characteristics that can affect elastomeric chains and the oral environment, such as the liquid temperature, a low pH, a high sugar content, artificial coloring, and alcohol content. Coca-Cola® was chosen, as it is a very popular carbonated soft drink (CSD) globally, and has a low pH and a large amount of sugar.[6] Coffee is a very popular drink with a low pH that is usually consumed at about 80°C, and can be used to observe the effects of heat and acidity on elastomeric chains. Alcohol can also affect the chains,[7] and beer was chosen as a drink with a low alcohol concentration for this study. The present in vitro study aimed to evaluate the force decay and elongation of 2 orthodontic chains exposed to different immersion media.

Material and methods

Groups

Group A (n = 15) used a thermoset closed chain – Generation II Power Chain (Part No./Ref 639-0002; Ormco, Brea, USA).

Group B (n = 15) used a Dentsply Raintree closed chain (SKU:300-044; Borgatta, Ixtapaluca, Mexico).

A diagram of the groups is shown in Fig. 1.

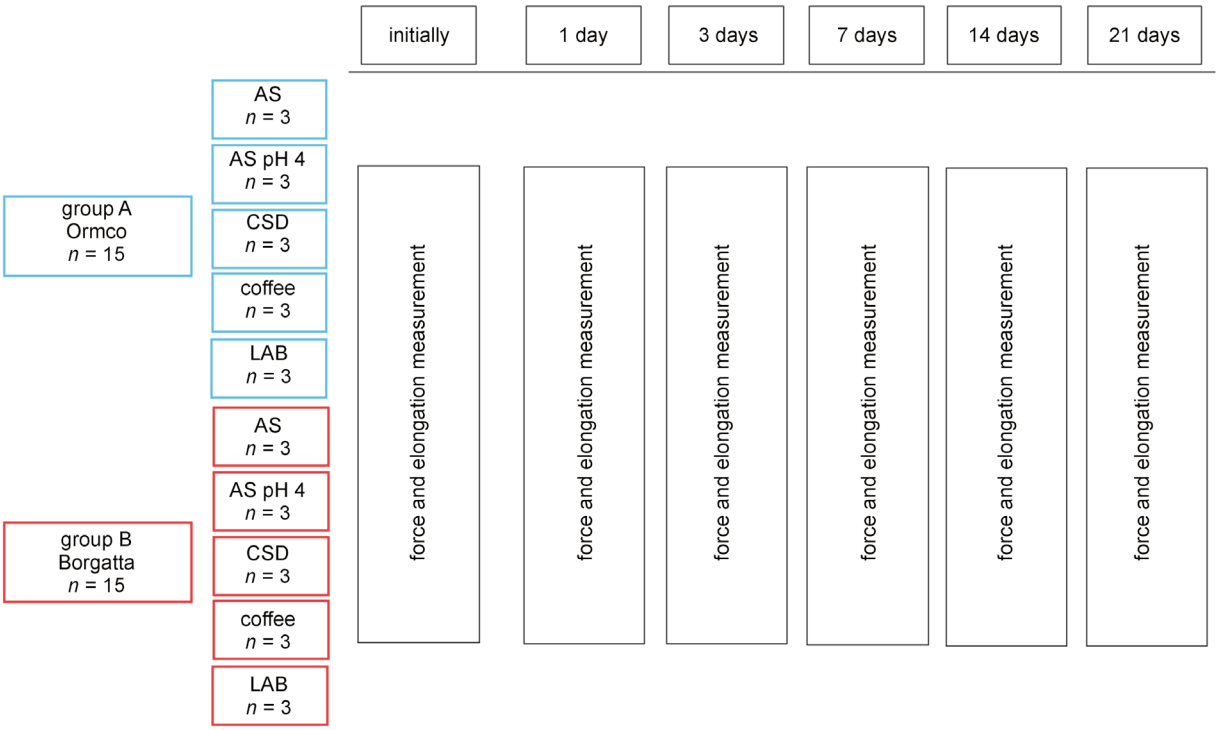

Fig. 1. Conformation of study groups

Group A – closed chain Ormco; group B – closed chain Dentsply, Borgatta. Both groups with 5 subgroups: artificial saliva (AS); AS at pH 4.0; carbonated soft drink (CSD); coffee, and low-alcohol beverage (LAB). The measurements of force decay and elongation were obtained at 0, 1, 3, 7, 14, and 21 days.

Exposure

The control chains were immersed in artificial saliva (AS) at 37°C, without exposure to other solutions. On the other hand, the experimental groups were immersed in AS at pH 4.0 (37°C) to simulate changes in saliva acidity following the consumption of beverages. The experimental groups also included chains immersed in common beverages to simulate daily consumption, including a CSD (Coca-Cola at a refrigeration temperature of about 4°C), coffee (freshly prepared using ground coffee beans in a conventional coffee maker, at a temperature of about 80°C) and a low-alcohol beverage (LAB) (beer at a refrigeration temperature of about 4°C), for 15 min every day, as shown in Fig. 1.

Procedure

Roth 0.22-inch slot premolar brackets (REM Orthodontics, Veracruz, Mexico) were cemented on acrylic plates with dimensions of 25 mm, 10 mm and 3 mm in length, width and thickness, respectively, using a previously worn surface. Following the manufacturer's instructions for each material used, a layer of silane and a layer of Tetric® N-Bond (Ivoclar Vivadent, Schaan, Liechtenstein) were applied, with the latter one light-cured; finally, a portion of Tetric EvoFlow fluid resin (Ivoclar Vivadent) was applied and light-cured. The brackets were placed 10 mm from center to center to emulate canine retraction, with sliding mechanics used to close premolar extraction sites. After evaluating the integrity of cementation, each chain was placed on the bracket and dragged to another bracket on the tension plate. The chains consisted of 3 loops (unit modules) – 2 attached to the brackets and 1 free loop in the middle. The chains were immersed in the different liquid environments for 15 min every day, for 21 days. For the rest of the day, the chains were placed into 10 mL of AS (Viarden, Mexico City, Mexico) in 6-well microplates (Costar® Cell Culture Plate Non-Pyrogenic, model 3506; Corning Life Sciences, Kennebunk, USA) and stored in an incubator at 37°C. The control group chains were also immersed in AS at 37°C the entire time. The AS was replaced every 3 days in all groups to compensate for the liquid evaporation caused by the incubation temperature. After each exposure, the samples were rinsed with 15 mL of sterile saline solution.

The force was measured in grams [g], using a Dontrix tensiometer force gauge (Morelli Ortodontia, Sao Paulo, Brazil). The length measurements of the orthodontic chains were performed with high-precision digital Vernier calipers (Morelli Ortodontia) to determine elongation. The measurements were carried out at the following time intervals: baseline (before placing on the tension plate), and 1 day, 3 days, 7 days, 14 days, and 21 days after the first immersion, as shown in Fig. 1.

To maintain blinding, one operator placed the chains and made the measurements, while another analyzed the data without knowing the nomenclature of each group.

Statistical analysis

Descriptive data were expressed as mean ± standard deviation ($M \pm SD$). The Shapiro–Wilk normality test was performed for all data, and the statistical analysis used the Mann–Whitney U test. The IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA), was used for the analysis. Differences were statistically significant at $p \leq 0.05$.

Results

The normality test showed that the force decay and chain elongation data did not follow a normal distribution. The force decay and elongation results are shown in Table 1 and Table 2, respectively, and in Fig. 2.

Table 1. Decay in the force [cN] of the elastomeric chains exposed to different liquid environments, evaluated at 6 different time intervals ($n = 3$)

Liquid	Group	Time intervals					
		initially	1 day	3 days	7 days	14 days	21 days
AS	group A	221.63 ±1.07^{a}	140.52 ±5.58^{Ab}	120.91 ±2.84^{Ac}	111.10 ±12.35^{Ac}	89.82 ±14.02^{Ad}	76.78 ±7.45^{Ae}
	group B	416.78 ±0.88^{a}	155.23 ±28.24^{Ab}	116.01 ±7.45^{Ac}	107.87 ±2.64^{Ac}	73.54 ±24.51^{Ad}	60.40 ±12.35^{Ad}
AS pH 4	group A	220.64 ±2.94^{a}	132.38 ±2.05^{Bb}	122.58 ±2.05^{Ac}	106.20 ±14.12^{Ad}	75.11 ±2.84^{Be}	73.54 ±5.09^{Ae}
	group B	441.29 ±1.17^{a}	171.61 ±3.13^{Bb}	130.72 ±14.12^{Bc}	127.48 ±8.43^{Ac}	89.63 ±14.12^{Bd}	62.07 ±11.27^{Ae}
CSD	group A	224.57 ±1.07^{a}	155.23 ±14.12^{Ab}	140.52 ±50.30^{Ab}	114.34 ±14.02^{Ac}	89.92 ±14.12^{Ac}	86.59 ±11.27^{Ad}
	group B	433.06 ±14.02^{a}	196.13 ±49.03^{ABb}	158.47 ±53.93^{Bbc}	116.01 ±14.12^{Ac}	73.54 ±3.23^{Ad}	63.74 ±4.90^{Ae}
Coffee	group A	228.59 ±14.12^{a}	147.09 ±1.96^{Cb}	127.48 ±8.43^{Ac}	112.77 ±8.43^{Ac}	94.73 ±5.58^{Ad}	75.11 ±2.84^{Ae}
	group B	473.95 ±14.02^{a}	147.09 ±1.47^{Ab}	150.33 ±5.58^{Bb}	135.62 ±10.29^{Ac}	103.3 ±5.58^{Cd}	80.02 ±11.27^{Ae}
LAB	group A	228.78 ±14.31^{a}	147.09 ±1.96^{Cb}	127.48 ±8.43^{Ac}	112.77 ±8.43^{Ad}	94.73 ±5.47^{Ae}	75.11 ±2.84^{Af}
	group B	392.26 ±2.25^{a}	152.0 ±8.43^{Ab}	116.01 ±15.78^{Ac}	106.20 ±14.12^{Ac}	49.03 ±3.92^{Dd}	37.55 ±12.35^{Be}

Data presented as mean ± standard deviation ($M \pm SD$). Different uppercase letters indicate statistical differences within the groups with regard to different liquid environments, whereas different lowercase letters indicate statistical differences between the samples across the time intervals ($p < 0.05$; Mann–Whitney test).

Table 2. Elongation [mm] of the elastomeric chains exposed to different liquid environments, evaluated at 6 different time intervals ($n = 3$)

Liquid	Group	Time intervals					
		initially	1 day	3 days	7 days	14 days	21 days
AS	group A	6.0 ±0.10^{a}	6.8 ±0.03^{Ab}	7.3 ±0.04^{Ac}	7.3 ±0.03^{Ac}	7.4 ±0.03^{Ad}	7.6 ±0.11^{Ae}
	group B	5.5 ±0.02^{a}	6.5 ±0.16^{Ab}	7.0 ±0.12^{Bc}	7.5 ±0.16^{Ad}	7.9 ±0.05^{Ae}	8.4 ±0.16^{Af}
AS pH 4	group A	6.0 ±0.12^{a}	6.2 ±0.04^{Bb}	6.3 ±0.07^{Bc}	6.6 ±0.03^{Bd}	6.7 ±0.13^{Bd}	7.4 ±0.16^{Ae}
	group B	5.5 ±0.04^{a}	6.6 ±0.04^{Ab}	7.2 ±0.29^{ABc}	7.3 ±0.21^{ABc}	7.7 ±0.23^{Ad}	8.5 ±0.06^{Ae}
CSD	group A	6.0 ±0.03^{a}	6.8 ±0.06^{Ab}	6.8 ±0.06^{Cb}	7.1 ±0.13^{CDc}	7.4 ±0.17^{Ad}	7.4 ±0.21^{Ad}
	group B	5.5 ±0.02^{a}	6.6 ±0.06^{Ab}	7.3 ±0.15^{Ac}	7.6 ±0.06^{Bd}	7.9 ±0.08^{Ae}	8.2 ±0.17^{Af}
Coffee	group A	6.0 ±0.10^{a}	6.7 ±0.10^{Cb}	7.0 ±0.10^{Dc}	7.0 ±0.02^{Cc}	7.1 ±0.13^{Dc}	7.2 ±0.06^{Bd}
	group B	5.5 ±0.05^{a}	6.5 ±0.20^{Ab}	6.7 ±0.12^{Db}	7.5 ±0.06^{Ac}	7.9 ±0.04^{Ad}	8.3 ±0.28^{Ae}
LAB	group A	6.0 ±0.01^{a}	6.6 ±0.16^{Cb}	7.0 ±0.01^{Dc}	7.2 ±0.09^{Dd}	7.3 ±0.07^{Ad}	7.4 ±0.04^{Cd}
	group B	5.5 ±0.02^{a}	6.6 ±0.10^{Ab}	7.0 ±0.03^{Bb}	7.6 ±0.25^{Bc}	8.1 ±0.38^{Bd}	8.8 ±0.65^{Ae}

Data presented as *M* ±*SD*. Different uppercase letters indicate statistical differences within the groups with regard to different liquid environments, whereas different lowercase letters indicate statistical differences between the samples across the time intervals ($p < 0.05$; Mann–Whitney test).

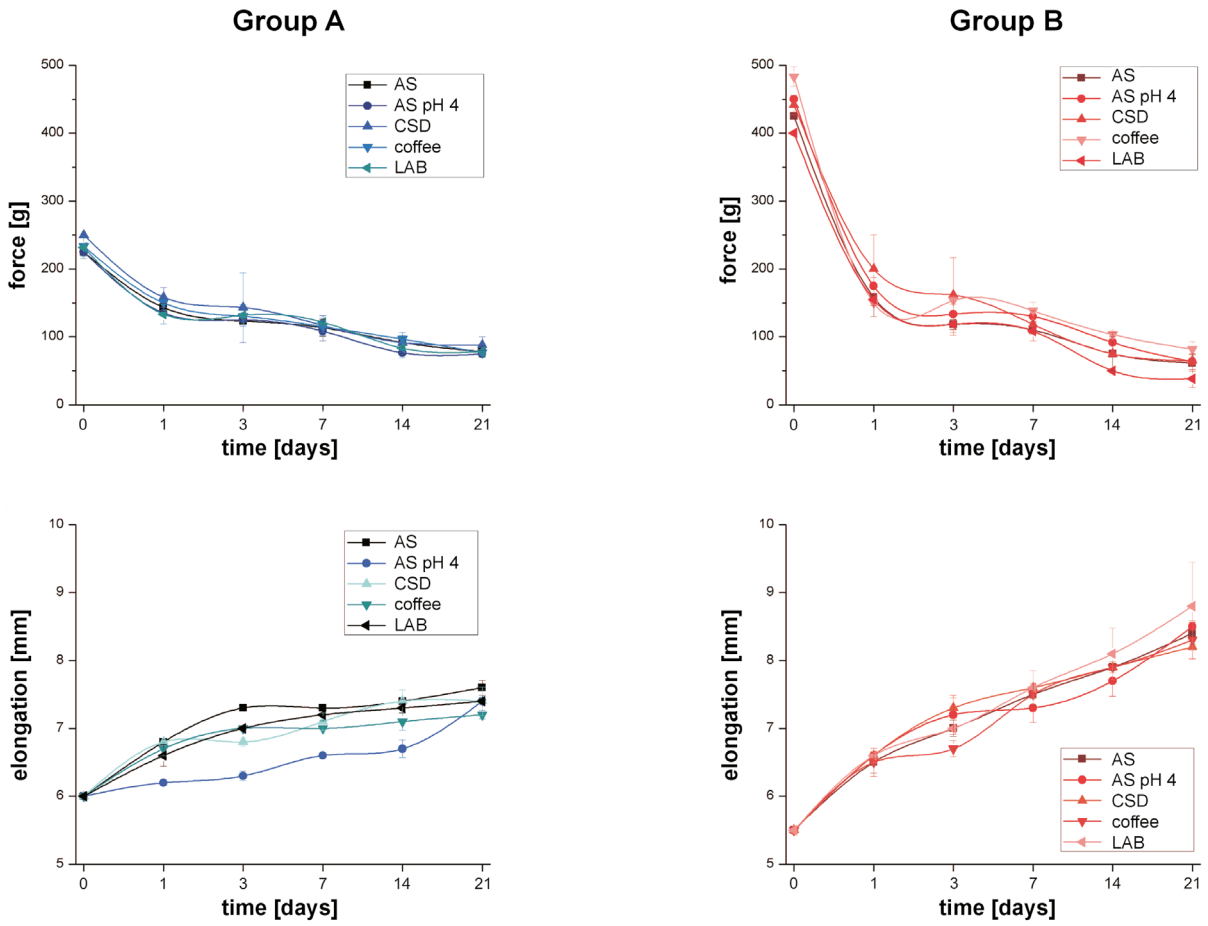

Fig. 2. Graph representing the force decay and elongation of the 2 groups of elastomeric chains exposed to different liquid environments

The group A chains immersed in AS at pH 4.0 (z = –1.993; 95% confidence interval (*CI*): –11.3–17.9; $p = 0.561$), CSD (z = –1.107; 95% *CI*: –32.2–12.2; $p = 0.268$), coffee (z = –0.449; 95% *CI*: 11.4–14.8; $p = 0.653$), and LAB (z = –0.449; 95% *CI*: 11.4–14.8; $p = 0.653$) exhibited comparable values of force at the end of the follow-up, and there were no significant differences as compared to the control (Table 1).

Group B showed the dispersion of the data with regard to the initial force of the chains. In addition, in the intragroup comparisons, the chains lost a large percentage of strength at the end of the follow-up in relation to base-

line in all liquid environments. The group B immersed in AS at pH 4.0 (z = 0.346; 95% *CI*:= –29.0–25.7; p = 0.692), CSD (z = –0.443; 95% *CI*: –25.0–18.4; p = 0.658) and coffee (z = –1.623; 95% *CI*: –47.4–7.4; p = 0.105) exhibited similar force values to the control at the end of the follow-up. At the same time interval, the LAB group had the lowest force value (z = –23.300; 95% *CI*: 5.2–51.9; p = 0.050) in comparison with the control, which has clinical relevance, since the exerted force could be insufficient (Table 1).

The group B chains showed higher force decay and elongation at the end of follow-up as compared to the group A chains. At the initial measurement, the chains in group A had a greater size than the group B chains. At the end of the follow-up, no significant elongation differences were observed in the group A chains exposed to AS at pH 4.0 (z = –1.528; 95% *CI*: = –0.1–0.5; p = 0.127) and CSD (z = –1.091; 95% *CI*: –0.2–0.5; p = 0.275) as compared to the control. The chains exposed to coffee (z = –1.091; 95% *CI*: 0.10–0.6; p = 0.016) and LAB (z = –1.993; 95% *CI*: 0.0–0.4; p = 0.046) showed less elongation. From group B, the chains immersed in LAB presented higher elongation (z = –0.886; 95% *CI*: –1.5–0.7; p = 0.376), but the difference was not statistically significant (Table 2).

The group A chains immersed in AS had a more irregular surface than the group B chains, with micrographs (×1,500 magnification) showing superficial cracks on the chains. The chains in groups A and B exposed to AS at pH 4.0, CSD and LAB were similar to those in the control group. In contrast, the group A chains exposed to coffee had more visible cracks than those of the control (Fig. 3 and 4).

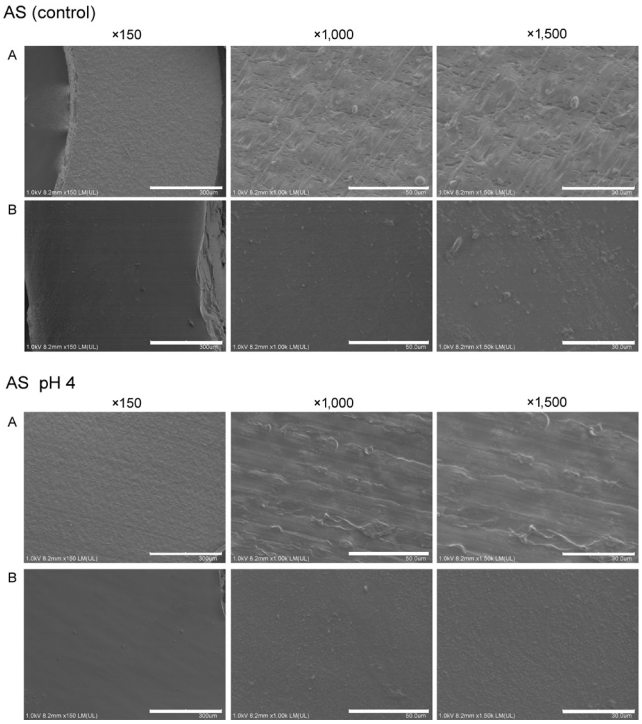

Fig. 3. Micrographs of elastomeric chains

Groups A (A) and B (B) after 21 days of exposure to AS (control) and AS at pH 4.0, at 3 magnifications – ×150, ×1,000 and ×1,500.

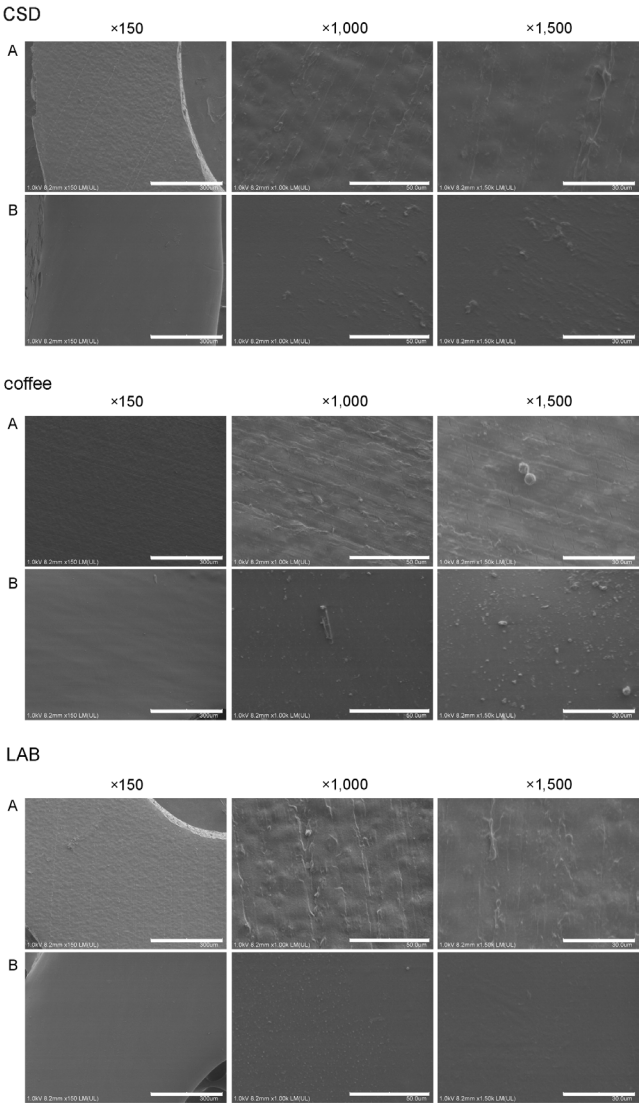

Fig. 4. Micrographs of elastomeric chains

Groups A (A) and B (B) after 21 days of exposure to CSD, coffee and LAB, at 3 magnifications – ×150, ×1,000 and ×1,500.

Discussion

In general, orthodontic elastomeric chains are made of polyurethanes, which are polymers synthesized from a polyaddition reaction between a diisocyanate or a polymeric isocyanate with a polyol in the presence of catalysts and additives. An unlimited number of these compounds can be found, with the only condition being the presence of a urethane group (-NH-CO-O-) in the polymer chain. The urethane group is obtained by the reaction between isocyanate and hydroxyl groups, although amines can be used in special cases. For polyurethane elastomers, the following can be obtained by reacting: a diisocyanate (aromatic or aliphatic; RN=C=O), a long-chain diol ($R-(OH)_2$), and a chain-extending low-molecular-weight diol or a diamine ($R-(NH)_2$). These polymers exhibit elastomeric behavior when possessing highly flexible chains, i.e., a low degree of intermolecular interaction

and the presence of cross-linked links, which prevent the sliding of the polymeric chains with others. The nature of the bonds can be physical or chemical; the difference between them is the melting or dissolution point of the resulting polymer. Chemical bonds produce an irreversible network that is difficult to destroy by using heat treatment, while physical bonds allow the melting or dissolution of the material.[8] Also, the mechanical properties of polyurethane elastomers can be modified by terminal urethane bonds in the copolymer. These elastomers are capable of forming hydrogen bonds with the functional groups of the neighboring molecules, creating a physical grid. Due to this, environmental factors (high temperatures, oxygen concentration, changes in pH, and water absorption, among others) affect the physical properties of elastomeric chains.[9,10]

In the present study, important differences were found in the initial force exerted by the chains at the baseline. This finding has been previously reported and attributed to dimensional variations of the elastomeric chain, even within the same brand.[11] Regarding their appearance, elastomeric chains can be transparent or colored, with the former used in the current study. It has been postulated that the pigments used in colored chains could contaminate the polymer and cause a decrease in the exerted force.[11] It seems that certain colors from a particular manufacturer showed a decrease in the force applied to the tooth; however, the magnitude of such a decrease does not appear to be clinically relevant.[12] The forces exerted by these materials are unstable and change over time depending on several factors, including the chain configuration (open or closed) and if the chain is pre-stretched, though this varies according to the speed and amount of stretch. Factors in the oral environment include saliva properties, such as changes in pH, and exposure to light, air, water, oxidants, food, and the rinses used in hygiene,[13] as well the application of forces during chewing and brushing the teeth, and the active substances of dentifrices. The effects of the bleaching agents of dentifrices on force decay in elastomeric chains have been reported, with the Sensodyne and Crest whitening pastes showing a greater loss of strength at 28 days than a standard dentifrice,[14] but the effects of other pastes containing arginine,[15] used for the prevention of caries or tooth sensitivity, on elastomeric chains have not been investigated.

In the present study, the chains were not pre-stretched and were placed using brackets to emulate the clinical conditions. In the same sense, the purpose of exposure to different liquid environments was to simulate a moderate daily consumption of beverages or a low-pH (pH 4.0) salivary environment. At 21 days, the resulting percentage of force exerted by the group A chains immersed in AS was 34%, whereas for the group B chains, it was 14% under the same conditions. In a study performed by Kassir et al., the authors reported 79.8% of force remaining in the chains of the same brand as used in group A; however, they used pins instead of brackets to place the chains.[16] As far as we know, there is no study on group B chains.

The results of the present study align with those of Teixeira et al., who demonstrated that strength reduction was more critical in the first 24 h.[17] Furthermore, no statistical difference was found after the exposure of the tested elastomeric chains to different acidic environments.[17]

The CSD showed less force decay for both groups (A and B) as compared to the control at 21 days, which is in accordance with the results reported by Kumar et al., who exposed the chains to a CSD for 1 min twice a day for 28 days.[18] Carbonated soft drinks contain carbonated water, water that contains carbonic acid (H_2CO_3), which is unstable and readily decomposes into water and carbon dioxide (CO_2). Carbonated water is responsible for the effervescence of these beverages. Although the release of oxygen could theoretically affect the elastomer bonds, the deterioration of the chains exposed to the CSD was not observed.

Regarding force decay due to the manufacturing process, thermoplastic and thermoset chains have been studied. Thermoplastics are made of a moldable polymer at high temperatures, whereas thermoset chains are irreversibly cured during the fabrication process. The group A chains were thermoset, and their mechanical behavior was more stable. However, the manufacturer of the group B chains did not provide information with regard to this. An in vitro study found that thermoplastic chains showed 20% more force decay when compared to the corresponding thermoset chains of the same brand.[19] In vivo, no differences were found between thermoplastic and thermoset chains.[20]

The current study found that coffee exposure did not result in increased force decay as compared to the control. Braga et al. investigated the effects of hot beverages (hot water, green tea and coffee) on the force decay of chains, and their results showed no statistical difference between the control and coffee groups at 21 days, in contrast with the other hot beverages.[21] On the other hand, Aldrees et al. studied the effects of coffee and found yellow discoloration of chains.[22]

The group B chains exposed to beer showed a higher decrease in force in comparison with the other fluids and the control. Group A did not show a difference in comparison with the control. Larrabee et al. tested the effects of alcohol exposure at concentrations of 14% and 26.9% on elastomeric chains, and their results showed that alcohol significantly decreased the force exerted by chains in a concentration-dependent manner.[23] The clinical relevance of the consumption of LABs on the force of elastomeric chains remains unclear.

The force required for orthodontic movement varies according to the type of movement. Low forces have been considered as those <100 cN and high forces as those >150 cN,[24] although there is still some controversy about

the optimal forces for each movement. In the case of canine retraction, 150 g of force is usually used.[25] However, other researchers have applied forces of 100 and 150 cN, and as a result, no significant differences in external apical root resorption and the movement rate were found.[26] In the present study, the magnitude of the force exerted by the chains at the end of the follow-up appears to be insufficient for canine retraction regardless of the type of liquid to which the chains were exposed.

A meta-analysis by Andhare et al. determined the proportion of difference in the force decay rate between in vitro and in vivo studies, and reported similar results from both types of studies, implying that in vitro research can replicate clinical conditions.[10]

Similar physical behavior has been observed in intermaxillary elastics. Large discrepancies have been shown in the physical and mechanical properties of elastics made by the same manufacturer regardless of the type of elastic.[27] Several in vitro studies have reported a greater strength loss in latex and non-latex intermaxillary elastics after placing them in the oral cavity.[28] Latex elastics presented superior strength than the non-latex ones.[29] Leão-Filho et al. evaluated the in vitro effects of frequently ingested beverages (Coca-Cola, coffee, orange juice, beer, red wine, and AS as the control) on the force degradation in intermaxillary elastics (1/4-inch).[30] As a result, they found no statistically significant differences between the beverages and concluded that the chemical nature of the beverages was not able to influence the degree of force degradation.[30] Likewise, in another study performed by Macedo et al., the authors did not find differences between the latex and non-latex elastics exposed to saliva and chlorhexidine solutions.[28]

Although the initial force that the chains exert on the teeth is sufficient, the changes occurring after 21 days may render them insufficient. Hence, the clinician needs to educate the patient on scheduling regular appointments. In addition, various strategies have been investigated as future perspectives for improving the efficiency of elastomeric chains. Nanotechnology has been used as a novel approach in dentistry to improve mechanical and biological properties.[31–33] In that sense, nanoparticles have also been developed for orthodontic materials[34,35] to avoid the reduction of elasticity, diminish surface alterations that occur due to the patient's diet[36] and provide antimicrobial activity. Furthermore, another approach consists in incorporating S-Nitroso-N-acetylpenicillamine, a synthetic nitric oxide, into the orthodontic elastomeric chain to generate an antibacterial polymer, which shows good short-term results.[37]

The present in vitro study did not fully mimic the oral conditions of the patient. The research was not intended to replicate the consumption of each of the most common drinks, since there are too many variables that intervene in the diet of each individual. However, it did compare the elastomeric chains subjected to different beverages to discover which of them produces a greater change in force decay and greater deformation.

Conclusions

Elastomeric chains suffer force decay and elongation as a function of time, mainly in the first 24 h. At the end of the follow-up, the group A chains exhibited less force decay than the group B chains. Furthermore, the qualitative observations showed that the chains in group A had a more irregular surface than the chains in group B.

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

Apical extrusion of debris when using OneCurve, ProTaper Next and TruNatomy in curved canals

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Abstract

Background. The extrusion of apical debris is related to various factors, and may be affected by variations in technique or instrumentation system. Although the extrusion cannot be completely prevented, it is crucial to minimize the amount of extruded material.

Objectives. The present study aimed to compare apical debris extrusion by the novel TruNatomy (TRN), OneCurve (OC) and ProTaper Next (PTN) instruments in curved root canals.

Material and methods. A total of 60 multi-rooted human mandibular molar teeth with moderate and severe curvature were selected and randomly divided into 3 groups. The root canals were prepared with the OC, TRN and PTN files. For collecting the debris extruded through the apical foramen, Eppendorf tubes were used. After the vaporizing period, the tubes were re-weighed, and the amount of the extruded debris was calculated by subtracting the initial weight from the final weight. Statistical analysis was performed with the Shapiro–Wilk and Kruskal–Wallis tests. The statistical significance level was set at $p < 0.05$.

Results. The least amount of debris was extruded with TRN and the greatest with PTN, but the difference between the groups was not significant ($p = 0.257$).

Conclusions. All instrumentation systems were associated with debris extrusion. The tested file systems presented similar results in terms of apical debris extrusion in curved canals. The novel TRN system demonstrated promising results, comparable to OC and PTN.

Keywords: One Curve, ProTaper Next, TruNatomy, apical extrusion, endodontic instrumentation

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Introduction

Mechanical preparation is one of the most critical factors for the success of root canal treatment. There are various nickel-titanium (NiTi) file types and systems available for mechanical preparation,[1] although the results concerning their use differ. The files may demonstrate some complications during treatment due to deformation. Numerous factors have been reported for NiTi file complications, such as the operator's skills/experience, the dynamics of the instrument use, the instrumentation technique, the number of uses, the instrument design, the anatomic configuration of the root canals, the alloy/metallurgy, and the number of sterilization cycles.[2] Manufacturers aim to improve the properties of files to overcome these problems. Thus, it has been concluded that the superelastic effect is favorable to the proper treatment of root canals.[3] The properties of files have an influence on the shaping ability, the canal-centering ability, dentinal cracks, apical transportation, preserving the original anatomy of the root canal, and apical extrusion.[4–6]

Mechanical preparation aims to remove pulp tissue, necrotic tissue remnants, debris, and microorganisms.[7] During instrumentation, dentin remnants, irrigants, necrotic tissues, microorganisms, and their by-products may be extruded apically. The apical extrusion of these materials can result in periapical inflammation and postoperative pain.[8] Although the extrusion cannot be prevented entirely, it is crucial to minimize the amount of extruded material.

Extrusion is related to the preparation techniques, the type of instruments and the number of files used for preparation.[9,10] Thus, variations in technique or instrumentation system may affect debris extrusion. The crown-down technique results in the least amount of extruded debris in comparison with other techniques.[11]

Root canal curvature is related to limitations during endodontic treatment, with the treatment of curved canals limited by the angle and radius of the curvature. The curvature may lead to some complications, including the fracture of the instrument, the loss of working length, apical transportation, and zipping.[12] However, Leonardi et al. found no significant difference in the amount of extruded debris between mild and moderate curvature.[13]

OneCurve (OC; Micro-Mega, Besançon, France) and ProTaper Next (PTN; Dentsply Maillefer, Ballaigues, Switzerland) are well-known NiTi rotary file systems. The PTN system is a full-sequence conventional system working with rotational movement. The PTN files are manufactured from a special alloy named the M-wire, with the use of proprietary heat treatment.[14] The OC system is based on a single instrument for preparation, which works with rotational movement, similar to PTN. Besides this similarity, the alloy of the OC files differs from the PTN system due to special heat treatment, and it is named the C-wire.[15]

TruNatomy (TRN; Dentsply Sirona, Ballaigues, Switzerland) has been recently developed as a heat-treated NiTi instrument with a different design. The TRN system consists of 3 files of different sizes, including small (20/0.04), prime (26/0.04) and medium (36/0.03).[16] The file surface provides more space for debris accumulation in the coronal direction.[17] Also, the files are more flexible and fatigue-resistant due to their design and heat treatment.

To date, no information is available on the efficacy of the novel TRN files in terms of apical debris extrusion. Therefore, the present study aimed to compare the OC, PTN and TRN files with regard to extruding debris in curved canals.

Material and methods

Sample collection

A total of 60 multi-rooted human mandibular molars with a fully formed apex were used for the study. Tissue remnants and calculus were removed mechanically. Teeth with complicated canal anatomy, immature root formation, internal or external resorption, and previous root canal treatment were excluded. The presence of a single apical foramen for each canal was determined under a dental operating microscope (Leica Microsystems, Wetzlar, Germany).

Apical patency was established for the mesiobuccal root canals of multi-rooted teeth with a #10 K hand file (Dentsply Maillefer). After sectioning the crowns 2 mm to the cementoenamel junction (CEJ) with a diamond disk, standard root samples of the same length were taken. The working length of each canal was determined 1 mm of the root apex. The canal curvature angles and radii of the untreated mesiobuccal root canals were calculated using ImageJ 1.48v (National Institutes of Health, Bethesda, USA) and evaluated according to the method described by Schneider.[18] Only the curvatures with angles of 25–40° and radii ≤10 mm were used. Distal roots were separated and removed. The specimens were numbered, and 3 equal groups (n = 20) were randomly created before the preparation of the root canals.

Experimental setup

The experimental design defined by Myers and Montgomery (1991) was selected to collect the apically extruded debris. The setup consisted of an Eppendorf tube, a rubber stopper that stabilized the root during preparation and a glass vial (Fig. 1). The teeth were placed into the stopper at the CEJ level and fixed with cyanoacrylate Pattex Super Glue (Türk Henkel, Istanbul, Turkey) to prevent solution leakage. The tube into which the debris and irrigants were collected was placed in the glass vial. A 27-gauge needle was placed in the system, within the stopper, to balance internal and external pressure.

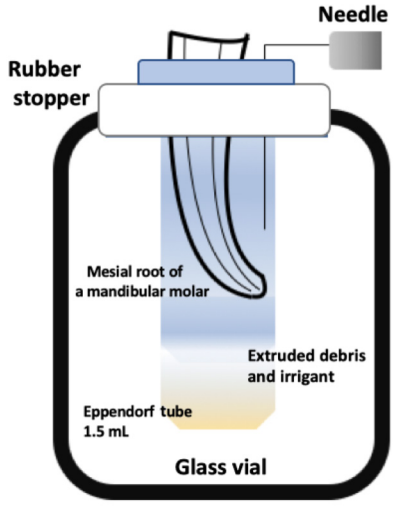

Fig. 1. Representative illustration of the experimental setup, as defined by Myers and Montgomery (1991)

Before preparation, the initial weight of the tubes was measured by using an analytical scale (RADWAG, Radom, Poland) with a precision of 10^{-4} g. Each Eppendorf tube was measured 3 times and the values were averaged. The glass vial was covered with an aluminum leaf to prevent the operator from seeing the apical foramen during preparation.

Root canal preparation

In total, 4 mL of distilled water was used for irrigation in each group during the preparation procedures. All files were used with the same X-Smart Plus endodontic motor (Dentsply Maillefer) according to the manufacturer's recommendations (Table 1).

Group 1: The OC files were used at 300 rpm speed and 2.5 N·cm torque settings. The path file was used for the initial preparation, and then the OC (25/0.06) files were used for the final preparation.

Group 2: The TRN files were used at 500 rpm speed and 1.5 N·cm torque settings. After the path file, the 20/0.04 (small) and 26/0.04 (prime) files were used for instrumentation.

Group 3: The PTN files were used at 300 rpm speed and 2 N·cm torque settings. The X1 file (17/0.04) was followed by the X2 file (25/0.06) in a brushing outstroke movement.

Evaluation of apical extrusion

The needle, the stopper and the tooth were removed from the tube after the instrumentation was completed. For each specimen, 1 mL of distilled water was used to collect the debris accumulated on the root surface. The distilled water was evaporated in an incubator at 70°C for 5 days to obtain dry debris. The tubes containing the dry debris were re-weighed with the same balance. As in the previous measurement, the samples were weighed 3 times and the values were averaged.

Table 1. Experimental groups used in the study

Group	Number of files used	File size	Operational speed [rpm]	Torque [N·cm]
1 OC	1	25/0.06	300	2.5
2 TRN	2	20/0.04 and 26/0.04	500	1.5
3 PTN	2	17/0.04 and 25/0.06	300	2

OC – OneCurve; TRN – TruNatomy; PTN – ProTaper Next.

The amount of apically extruded debris was calculated by subtracting the weight of the empty tube from the weight of the tube containing the accumulated debris.

Statistical analysis

The statistical analysis employed the IBM SPSS Statistics for Windows software, v. 19.0 (IBM Corp., Armonk, USA). Data was presented as mean and standard deviation ($M \pm SD$). First, the data was analyzed using the Shapiro–Wilk test to verify the assumption of normality. The groups were then compared using the Kruskal–Wallis test for all variables. A p-value of less than 0.05 was considered statistically significant.

Results

There was no significant difference between the groups ($p = 0.270$). According to the mean values, the TRN group demonstrated the lowest, while the PTN group demonstrated the highest debris extrusion ($p = 0.257$). The mean, standard deviation, median, and minimum–maximum values are presented in Table 2 and Fig. 2.

Table 2. Comparison of the 3 groups in terms of apically extruded debris [g]

Group	$M \pm SD$	Me	min	max
OC	0.00064 ±0.00026	0.00055	0.00029	0.00112
TRN	0.00059 ±0.00025	0.00054	0.00027	0.00110
PTN	0.00072 ±0.00030	0.00073	0.00027	0.00114

M – mean; *SD* – standard deviation; *Me* – median; min – minimum; max – maximum. $p = 0.270$.

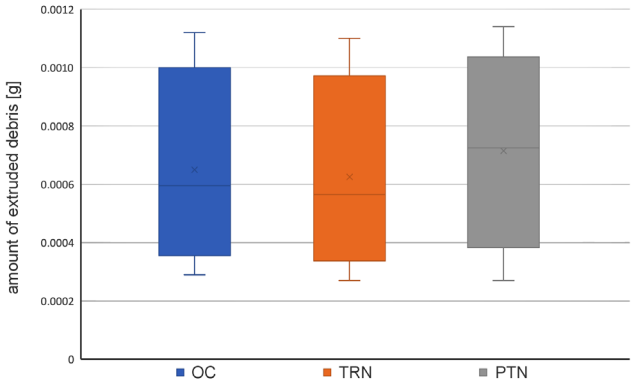

Fig. 2. Comparison of the amount of apically extruded debris in the 3 groups

Discussion

Apically extruded debris can cause several postoperative complications, such as inflammation and postoperative pain.[19] Therefore, a reduction in debris extrusion during root canal treatment may positively affect the postoperative conditions. The preparation system used affects the amount of extruded debris, while the methodology used to collect the debris has a limitation of simulating periapical tissues. According to Versiani et al., a standard comparison can be made by providing the same conditions, which can be achieved in vitro in a laboratory.[20] Thus, it may be claimed that the main advantage of the debris collection method used in the present study was standardization.

Distilled water has been proposed as an irrigation solution instead of sodium hypochlorite (NaOCl) so as not to affect the measurements, since the sodium crystals cannot be removed from the debris.[21] Tinaz et al. reported that the width of the apical structure could change the results regarding the apically extruded debris.[10] To avoid these disadvantages and to standardize the samples in all groups, apical patency was established with a #10 K file. The incidence of postoperative pain due to apical debris extrusion is reportedly higher in curved canals.[22,23] Therefore, in the current study, the mesiobuccal canals of mandibular molar teeth, with moderate to severe curvatures, were selected to compare the 3 different NiTi file systems in terms of apical debris extrusion.

Various NiTi systems are available for the instrumentation of root canals during endodontic treatment, with PTN being a commonly used and accepted system. Various studies have reported the occurrence of debris extrusion after the instrumentation with the use of the PTN files.[19,24] Reddy and Hicks reported that the instrument design was crucial for the amount of apically extruded debris.[11] The design of PTN decreases the interaction between the file and dentin, and minimizes the removal of debris from the apex, which is the main advantage of PTN and may cause less apical debris extrusion.[21] The PTN files were associated with less extrusion in straight canals as compared to the Controlled Memory NiTi files.[24] However, a conflicting result was observed during the instrumentation of curved root canals, with the PTN files associated with significantly more extrusion.[25] This discrepancy reveals the relationship between the anatomy of the root and the amount of extruded debris. A microcomputed tomographic evaluation revealed that the PTN system provided suboptimal mechanical preparation for molar teeth and was unable to obtain completely packed debris-free root canal surfaces.[26] This conclusion may explain an increase in debris extrusion in posterior teeth, which are relatively difficult to prepare.

The OC system includes a single instrument for preparing the root canal. Reducing the number of files during preparation minimizes the contact area between the file and the dentin wall. Such a situation may be beneficial for reducing the apical extrusion of debris by accommodating more space for the debris around the file.[27] However, under the same conditions, no differences were noted between the OC single-file, 2Shape (Micro-Mega) and PTN systems in terms of apical debris extrusion.[28] Similarly, Bürklein et al. found no difference between single- and multiple-file systems for the apical extrusion of debris.[29] The results of the present study support these findings, as the OC single-file system demonstrated similar results to both the PTN and TRN multiple-rotary file systems. Thus, it can be concluded that the number of files did not significantly affect debris extrusion.

Recently, Tüfenkçi et al. reported that by preparing a contracted endodontic cavity, the OC system caused less apical debris extrusion than the reciprocating single-file system.[30] This finding was related to the C-wire heat treatment technology. The C-wire provides the OC files with enhanced flexibility, easy access to canals, and the ability to pre-bend in order to preserve the original form of the root canal during preparation.[31,32] This property may be beneficial for optimal mechanical preparation without unnecessarily removing additional dentin, which might lead to more debris accumulation beyond the apex, especially in curved canals.

The most important difference in the TRN design is the use of 0.8-millimeter NiTi wire instead of the 1.2-millimeter one. The increased flexibility may facilitate the file movement in the root canal during preparation. The special design of the TRN files creates a slim shape that provides more space for debridement,[16] while the lower tapered design may help preserve the tooth structure. Limited information is available about the novel TRN system, although cyclic fatigue studies demonstrated promising results for the resistance of the TRN system as compared to various NiTi files.[33,34] The present study demonstrated comparable results for the novel TRN files in relation to the commonly used PTN and OC systems. The lower taper of TRN may prove advantageous, especially in curved canals, by preventing damage to the dentinal structure, and may lead to the reduction of debris extrusion.

Limitations

The main limitation of the present study was that the experimental design could not mimic periapical tissues and their resistance. There are some materials used to imitate their textures. Agar gel and floral foam may be used for the periapical area. However, these materials also have limitations, such as difficulties in setting and achieving definite values for agar gel, and the absorption of the extruded material for the foam.[35,36] Another limitation of this study was the use of extracted teeth for the experiment. The standardization of the extracted root canals was difficult, especially those with curvature. However, using root canals manufactured from acrylic or plastic has

several adverse effects. Indeed, the heat generated during preparation might soften the simulated tooth, which could affect the results.[37]

Within the limitations, our laboratory experiments represent preliminary results for improving the clinical conditions. It is crucial to evaluate the new rotary file systems to minimize apical debris extrusion for the success of endodontic treatment and a comfortable postoperative process.

Conclusions

All instrumentation systems were associated with debris extrusion. The tested file systems presented similar results in terms of apical debris extrusion in curved canals. The novel TRN system demonstrated promising results, comparable OC and PTN.

Ethics approval and consent to participate

The study protocol was accomplished under the Ethics Committee's standing orders (protocol No. 2020-09-16/18).

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

Implant practitioners' knowledge, awareness and attitudes regarding soft and hard tissue considerations at a single implant site: A questionnaire study

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Abstract

Background. The success of implant therapy depends on the proper evaluation of soft and hard tissues around implants, and the careful selection of biomaterials to manage the associated defects. To attain healthy peri-implant tissues, knowledge about evaluating and managing soft and hard tissues around dental implants is essential.

Objectives. The aim of the present study was to assess the knowledge, awareness and attitudes regarding soft and hard tissue considerations for single implant sites among the registered dental implant practitioners.

Material and methods. The current survey was carried out among dental implant practitioners registered with the Indian Dental Association (IDA). A total of 49 specialists practicing implant dentistry for at least 1 year were included in the study. The survey was conducted using a census approach. A validated questionnaire was circulated digitally among the participants, with 3 rounds of follow-up. To fill in the questionnaire, the participants' consent was required.

Results. The study involved 14 general practitioners (GPs), 7 periodontists, 10 oral and maxillofacial surgeons (OMFSs), 13 prosthodontists, and 5 certified implantologists. The majority of the participants (93.9%) felt that the soft tissue biotype would influence the treatment outcome ($p < 0.05$). Most of them (91.8%) evaluated the width of hard tissue at the implant site before surgery ($p > 0.05$), but only 77.6% of the population evaluated the bone width while considering the future prosthesis ($p > 0.05$). Although all participants placed implants, only 46.9% performed soft tissue augmentation by themselves ($p < 0.001$), whereas in the case of hard tissue augmentation, the percentage was 65.3% ($p < 0.001$).

Conclusions. The study identified various knowledge gaps among different practitioners with different educational background. The educational background seemed to have played a significant role in their attitude toward the evaluation and management of soft and hard tissues around dental implants.

Keywords: CDE programs, dental surgeons, dental association, dental professionals, general practitioners

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Introduction

Dental implants have become a predictable alternative for the replacement of missing natural teeth.[1] In the early 1980s, implants were mainly placed and managed by oral surgeons and periodontists, but today, implant dentistry is considered an interdisciplinary branch that requires sound knowledge of both surgical and prosthetic aspects.[2] However, various organizations and research institutes have introduced certified programs for general practicing dentists willing to start their careers in implant dentistry over some time. With the introduction of such programs, implant placement is practiced by various dental practitioners, including prosthodontists, certified implantologists and general practitioners (GPs).

The success of implants, placed with the use of various protocols, has increased, with predictable techniques and a better understanding of soft and hard tissue biology around implants.[3] However, the number of complications has also increased significantly with the increasing number of implants being placed. Therefore, determining implant success has changed significantly from earlier concepts, which focused only on peri-implant tissues,[4] to the latest approaches, which include patient-related outcomes, such as esthetics.[5,6] As such, there have been many factors discussed in the literature that can affect peri-implant health and long-term clinical outcomes.[7] They include the type of implant–abutment connection,[8] the type of retention (cemented or screw-retained),[9] soft tissue considerations, such as the tissue biotype, the width of keratinized gingiva (WKG),[10] and hard tissue considerations, such as the buccal bone thickness and/or the width of the available bone.[11]

The majority of those factors are directly related to the proper evaluation of soft and hard tissues around implants, and the careful selection of biomaterials to manage deficiencies. The challenge lies in successfully manipulating soft and hard tissues around the implant for positive long-term results. To achieve optimal clinical and subclinical peri-implant health,[12,13] it is indispensable to have knowledge about the evaluation of soft and hard tissues around dental implants. However, studies assessing such knowledge in implant practitioners are scant. Hence, the present study evaluated the knowledge, awareness and opinions of the registered dental practitioners regarding soft and hard tissue considerations around single dental implant sites based on the available evidence.

Material and methods

The present study was a questionnaire-based cross-sectional survey carried out among implant practitioners registered with the Indian Dental Association Dakshina Kannada (IDA-DK). Implant practitioner data was collected from the IDA-DK branch. After obtaining the approval of the institutional ethics committee (Ref. No. ETHICS/ABSMIDS/135/2021), an online questionnaire was sent to the dental practitioners between August and October 2021.

The survey was carried out anonymously, using a census approach, wherein all willing participants were included, and those unwilling were excluded. The selection criterion was dental practitioners with at least 1 year of practice in implant dentistry. Data from a total of 106 practitioners was collected, out of which 58 were identified as implant dentistry practitioners. Seven of the 58 were not interested in participating, 1 acted as an expert for survey validation and 1 was a part of the study (S.B.S.), leaving 49 respondents.

A self-administered questionnaire was sent to the participants digitally, with three rounds of follow-up. The purpose of the study was explained in detail, and confidentiality regarding their participation was assured.

The objective of the study was to:

– assess the knowledge regarding soft and hard tissue evaluation at single implant sites;
– assess the awareness regarding soft and hard tissue augmentation techniques at single implant sites;
– understand the clinicians' attitudes regarding the selection of soft and hard tissue augmentation techniques at single implant sites.

The questionnaire was written in English for easy perception and response, and contained precise questions on the topic. However, no question on the source of the participants' knowledge was covered.

The questionnaire was divided into 3 sections: general information; soft tissue considerations; and hard tissue considerations.

Statistical analysis

All data was obtained from Google Forms, entered into a Microsoft Excel file, verified, validated, and then analyzed using IBM SPSS Statistics for Windows, v. 23 (IBM Corp., Armonk, USA). The hypothesis was tested using qualitative variables represented by percentages and with the χ^2 test. A p-value <0.05 was considered statistically significant when the data was analyzed at the 95% confidence interval (*CI*) level.

Results

A total of 49 participants were involved in the study: 14 GPs; 7 periodontists; 10 oral and maxillofacial surgeons (OMFSs); 13 prosthodontists; and 5 certified implantologists (Table 1). The study results indicate that 63.3% of the participants had fewer than 5 years of experience, and 10.2% had more than 15 years of experience in implant dentistry. However, there was no statistical significance between the groups. Most of the

Table 1. Questionnaire data – general information

Variable		Types of practitioners					Total $N = 49$	*p*-value
		GPs $n = 14$	periodontists $n = 7$	OMFSs $n = 10$	prosthodontists $n = 13$	certified implantologists $n = 5$		
Experience in implant dentistry [years]	1–5	10 (71.4)	3 (42.9)	6 (60.0)	9 (69.2)	3 (60.0)	31 (63.3)	>0.05
	6–10	2 (14.3)	1 (14.3)	1 (10.0)	2 (15.4)	1 (20.0)	7 (14.3)	
	10–15	0 (0.0)	2 (28.6)	1 (10.0)	2 (15.4)	1 (20.0)	6 (12.2)	
	>15	2 (14.3)	1 (14.3)	2 (20.0)	0 (0.0)	0 (0.0)	5 (10.2)	
Sector of work	private	10 (71.4)	5 (71.4)	8 (80.0)	10 (76.9)	3 (60.0)	36 (73.5)	>0.05
	public	1 (7.1)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	2 (4.1)	
	both	3 (21.4)	2 (28.6)	2 (20.0)	2 (15.4)	2 (40.0)	11 (22.4)	
Affiliation with an educational institution, i.e., research or training	yes	6 (42.9)	4 (57.1)	7 (70.0)	6 (46.2)	3 (60.0)	26 (53.1)	>0.05
	no	8 (57.1)	3 (42.9)	3 (30.0)	7 (53.8)	2 (40.0)	23 (46.9)	
Did you attended a CPD/CDE course pertaining to implants in the last 2 years	yes	7 (50.0)	6 (85.7)	9 (90.0)	11 (84.6)	5 (100.0)	38 (77.6)	>0.05
	no	7 (50.0)	1 (14.3)	1 (10.0)	2 (15.4)	0 (0.0)	11 (22.4)	
With respect to the cost–benefit ratio, which mode of treatment do you advice?	implant	10 (71.4)	5 (71.4)	9 (90.0)	8 (61.5)	5 (100.0)	37 (75.5)	>0.05
	FPD	4 (28.6)	2 (28.6)	1 (10.0)	5 (38.5)	0 (0.0)	12 (24.5)	
Factors influencing implant selection	quality and quantity of the available bone	6 (42.9)	6 (85.7)	5 (50.0)	8 (61.5)	4 (80.0)	29 (59.2)	>0.05
	patients economic status/ implant cost	3 (21.4)	0 (0.0)	4 (40.0)	2 (15.4)	1 (20.0)	10 (20.4)	
	ease of prosthetic rehabilitation	1 (7.1)	1 (14.3)	1 (10.0)	2 (15.4)	0 (0.0)	5 (10.2)	
	micro-design	3 (21.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.1)	
	availability and support	1 (7.1)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	2 (4.1)	
Type of implant system	platform-switched	6 (42.9)	7 (100.0)	6 (60.0)	10 (76.9)	4 (80.0)	33 (67.3)	>0.05
	platform-matched	8 (57.1)	0 (0.0)	4 (40.0)	3 (23.1)	1 (20.0)	16 (32.7)	
Which factors influence your decision of choosing platform-switched implants?	available soft tissue	0 (0.0)	1 (14.3)	1 (16.7)	0 (0.0)	0 (0.0)	2 (6.1)	>0.05
	available hard tissue	1 (16.7)	1 (14.3)	2 (33.3)	2 (20.0)	3 (75.0)	9 (27.3)	
	available soft and hard tissues	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)	
	available soft and hard tissues, and esthetics	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	1 (3.0)	
	available soft tissue and esthetics	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	1 (3.3)	
	function and esthetics	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	1 (3.3)	
	function	1 (16.7)	2 (28.6)	1 (16.7)	1 (10.0)	0 (0.0)	5 (15.2)	
	esthetics	2 (33.3)	1 (14.3)	1 (16.7)	1 (10.0)	0 (0.0)	5 (15.2)	
	all	1 (16.7)	2 (28.6)	1 (16.7)	3 (30.0)	1 (25.0)	8 (24.2)	
Which factors influence your decision of choosing platform-matched implants?	available hard tissue	3 (37.5)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	4 (25.0)	>0.05
	available hard tissue and function	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	1 (6.3)	
	available soft and hard tissues	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	1 (6.3)	
	available hard tissue, function and esthetics	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.3)	
	function and esthetics	1 (12.5)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	2 (12.5)	
	function	1 (12.5)	0 (0.0)	1 (25.0)	1 (33.3)	0 (0.0)	3 (18.8)	
	all	2 (25.0)	0 (0.0)	1 (25.0)	0 (0.0)	1 (100.0)	4 (25.0)	

Data presented as number (percentage) (*n* (%)). GP – general practitioner; OMFS – oral and maxillofacial surgeon; CPD – continuing professional development; CDE – continuing dental education; FPD – fixed partial denture.

study population had a private practice (73.5%), while 22.4% worked in private and public sectors. More than half of the participants (53.1%) had undertaken research or training, while 77.6% had attended continuing professional development (CPD)/continuing dental education (CDE) courses on implants in the last 2 years. None of the certified implantologists had attended any CPD/CDE courses. In addition, 75.5% of the participants deemed implants to be a better treatment option than a fixed partial denture (FPD), and 38.5% of the prosthodontists deemed FPD superior to implants. The quality and quantity of the available bone determined implant selection among 59.2% of the participants, followed by the patient's economic status or the implant cost (20.4%). Around 67% of the participants favored the switched platform type, while the remaining preferred a platform matched to the type of implant placed. All periodontists and 76.9% of the prosthodontists preferred platform-switched implants, while 57.14% of GPs preferred platform matching (Table 1).

Table 2. Soft tissue considerations

Variable		Types of practitioners					Total $N = 49$	p-value
		GPs $n = 14$	periodontists $n = 7$	OMFSs $n = 10$	prosthodontists $n = 13$	certified implantologists $n = 5$		
Do you think that the soft tissue biotype influences your long-term treatment outcome?	yes	14 (100.0)	7 (100.0)	7 (70.0)	13 (100.0)	5 (100.0)	46 (93.9)	<0.05*
	no	0 (0.0)	0 (0.0)	3 (30.0)	0 (0.0)	0 (0.0)	3 (6.1)	
In which regions do you consider the soft tissue biotype?	in all cases	9 (64.3)	6 (85.7)	5 (71.4)	8 (61.5)	3 (60.0)	31 (67.4)	>0.05
	esthetic	3 (21.4)	1 (14.3)	2 (28.6)	4 (30.8)	2 (40.0)	12 (26.1)	
	functional	2 (14.3)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	3 (6.5)	
How do you evaluate the soft tissue biotype?	penetration with an endodontic file	1 (7.1)	3 (42.9)	0 (0.0)	2 (15.4)	1 (20.0)	7 (15.2)	>0.05
	color of the gingiva	2 (14.3)	1 (14.3)	2 (28.6)	1 (7.7)	0 (0.0)	6 (13.0)	
	penetration of with a probe at the edentulous site	5 (35.7)	1 (14.3)	3 (42.9)	4 (30.8)	4 (80.0)	17 (37.0)	
	probe transparency method on the adjacent tooth	6 (42.9)	2 (28.6)	2 (28.6)	6 (46.2)	0 (0.0)	16 (34.8)	
Do you think that WKG influences your long-term treatment outcome?	yes	8 (57.1)	7 (100.0)	6 (60.0)	10 (76.9)	2 (40.0)	33 (67.3)	>0.05
	no	6 (42.9)	0 (0.0)	4 (40.0)	3 (23.1)	3 (60.0)	16 (32.7)	
How do you evaluate the keratinized gingiva?	visual method	2 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	3 (9.1)	>0.05
	histochemical method by staining	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)	
	by using a probe to identify the mucogingival junction and by measuring the keratinized gingiva	4 (50.0)	6 (85.7)	6 (100.0)	10 (100.0)	1 (50.0)	27 (81.8)	
	visual and histochemical methods	0 (0.0)	1 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)	
	histochemical method and probe application	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)	
What, according to you, is the best material for enhancing the soft tissue biotype?	SCTG	3 (21.4)	7 (100.0)	4 (40.0)	6 (46.2)	1 (20.0)	21 (42.9)	>0.05
	FGG	5 (35.7)	0 (0.0)	2 (20.0)	4 (30.8)	1 (20.0)	12 (24.5)	
	soft tissue substitutes	6 (42.9)	0 (0.0)	4 (40.0)	3 (23.1)	3 (60.0)	16 (32.7)	
What, according to you, is the best material for increasing WKG?	SCTG	2 (14.3)	1 (14.3)	5 (50.0)	7 (53.9)	0 (0.0)	15 (30.6)	<0.05*
	FGG	3 (21.4)	5 (71.4)	1 (10.0)	2 (15.4)	2 (40.0)	13 (26.5)	
	collagen membrane	2 (14.3)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	3 (6.1)	
	enamel matrix derivative	0 (0.0)	1 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.0)	
	soft tissue substitutes	7 (50.0)	0 (0.0)	4 (40.0)	3 (23.1)	3 (60.0)	17 (34.7)	
In case of deficiency, do you perform the soft tissue augmentation procedures by yourself?	yes	4 (28.6)	7 (100.0)	8 (80.0)	2 (15.4)	2 (40.0)	23 (46.9)	<0.001*
	no, I call for a consultant	10 (71.4)	0 (0.0)	2 (20.0)	11 (84.6)	3 (60.0)	26 (53.1)	

Data presented as *n* (%). WKG – width of keratinized gingiva; SCTG – sub-epithelial connective tissue graft; FFG – free gingival graft; * statistically significant.

Most of the study population (93.9%) deemed the soft tissue biotype to influence the treatment outcome ($p < 0.05$), of which only 67.4% considered it in all cases. Meanwhile, 30% of the OMFSs thought that the soft tissue biotype had no influence on the treatment outcome. The majority of the population evaluated the soft tissue biotypes by using probe penetration at the edentulous site (37.0%) or the probe transparency method on the adjacent tooth (34.8%). Most of the participants (67.3%) and all periodontists considered WKG before implant placement. The keratinized gingiva was measured from the mucogingival junction to the gingival margin by 81.8% of the participants, including all OMFSs and prosthodontists. All periodontists and most of the OMFSs (80.0%) performed soft tissue augmentation by themselves, while the majority of the GPs (71.4%) and prosthodontists (84.6%) called for consultants ($p < 0.001$). All periodontists indicated the sub-epithelial connective tissue graft (SCTG) to be the best material for enhancing the soft tissue biotype. The SCTG was perceived to increase WKG the most among the participants (30.6%), followed by free gingival graft (FGG) (26.5%) ($p < 0.05$) (Table 2 and Fig. 1).

Most participants (91.8%), including all periodontists and OMFSs, evaluated the hard tissue width at the implant site before surgery, while 77.6% of the population evaluated the bone width while considering the future prosthesis, most being prosthodontists. The bone width was evaluated using cone-beam computed tomography (CBCT) by the majority of the participants (66.7%). Similarly, most of the practitioners (81.3%) used CBCT to evaluate the bone length, particularly the OMFSs (90.0%) and all prosthodontists ($p < 0.05$). The facial bone thickness was evaluated before implant placement by 83.7% of the population. Most of the participants (65.3%) considered contour augmentation during implant placement, while 18.4% performed it in all cases. Almost all participants (91.8%) considered augmenting the bone when the available amount of bone was less than in the case of regular platforms, while the remaining 8.7% chose narrow-platform or short implants in cases of deficiency. The majority of the participants performed hard tissue augmentation by themselves, including all periodontists and OMFSs ($p < 0.001$). Guided bone regeneration (GBR) was performed by 62.5% of the participants, while only 6.3% were familiar with all types of hard tissue augmentation techniques (Table 3 and Fig. 2).

Figure 3 presents the percentages of practitioners performing soft and hard tissue augmentation by themselves.

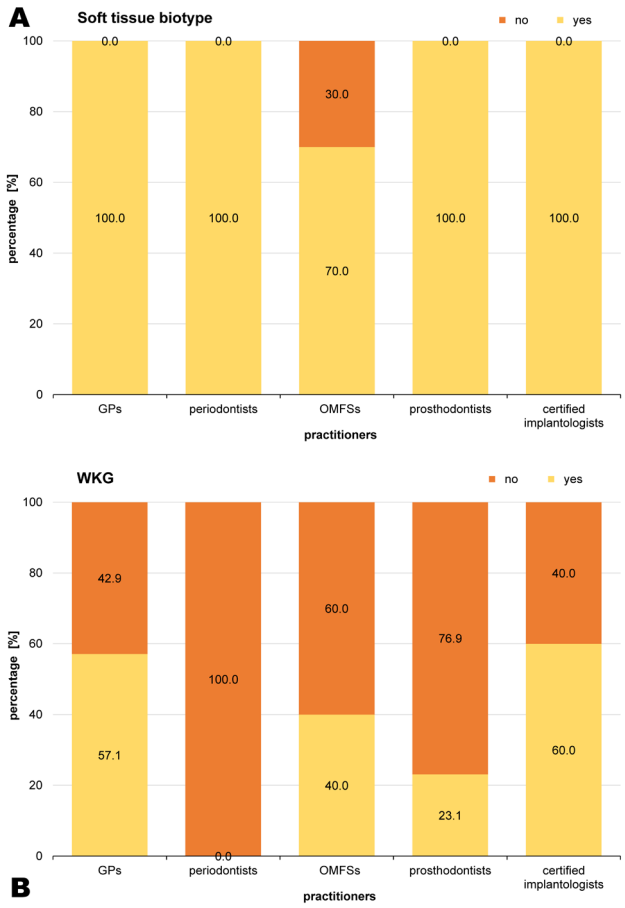

Fig. 1. Do you think the following parameter influences your long-term treatment outcome?

A – soft tissue biotype; B – WKG.

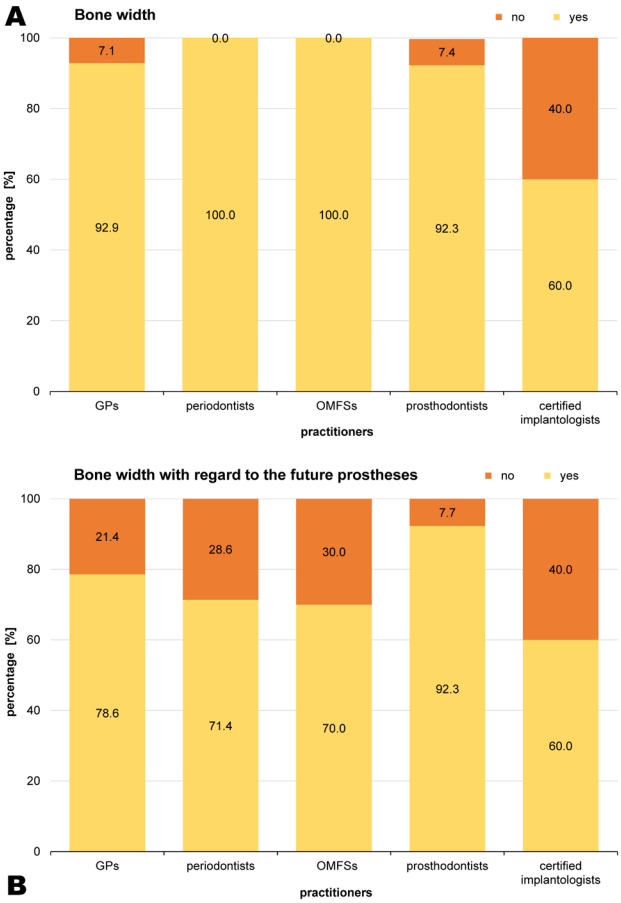

Fig. 2. Do you evaluate the following parameter?

A – bone width; B – bone width with regard to the future prostheses.

Table 3. Hard tissue considerations

Variable		Types of practitioners					Total $N = 49$	*p*-value
		GPs $n = 14$	periodontists $n = 7$	OMFSs $n = 10$	prosthodontists $n = 13$	certified implantologists $n = 5$		
Do you evaluate the available width of hard tissue at the implant site before surgery?	yes	13 (92.9)	7 (100.0)	10 (100.0)	12 (92.3)	3 (60.0)	45 (91.8)	>0.05
	no, I decide once the flap is reflected	1 (7.1)	0 (0.0)	0 (0.0)	1 (7.7)	2 (40.0)	4 (8.2)	
Which method do you use to evaluate the available width?	CBCT evaluation	8 (61.5)	4 (57.1)	9 (90.0)	8 (66.7)	1 (33.3)	30 (66.7)	>0.05
	clinically, by using a probe over the implant site	1 (7.7)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	2 (4.4)	
	by using a bone mapping caliper	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.4)	
	combination of the above	4 (30.8)	1 (14.3)	0 (0.0)	4 (33.3)	2 (66.7)	11 (24.4)	
Do you evaluate the bone width while considering the future prostheses?	yes	11 (78.6)	5 (71.46)	7 (70.0)	12 (92.3)	3 (60.0)	38 (77.6)	>0.05
	no, the prostheses are related to implant placement	3 (21.4)	2 (28.6)	3 (30.0)	1 (7.7)	2 (40.0)	11 (22.4)	
Do you evaluate the available length of the bone before surgery?	yes	14 (100.0)	7 (100.0)	10 (100.0)	13 (100.0)	4 (80.0)	48 (98.0)	>0.05
	no	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1(20.0)	1 (2.0)	
Which method do you use to evaluate the available length?	CBCT	11 (78.6)	5 (71.4)	9 (90.0)	13 (100.0)	1 (25.0)	39 (81.3)	<0.05*
	panoramic radiograph	3 (21.4)	2 (28.6)	1 (10.0)	0 (0.0)	3 (75.0)	9 (18.8)	
Do you consider the facial bone thickness prior to implant placement?	yes	12 (85.7)	6 (85.7)	9 (90.0)	10 (76.9)	4 (80.0)	41 (83.7)	>0.05
	no	2 (14.3)	1 (14.3)	1 (10.0)	3 (23.1)	1 (20.0)	8 (16.3)	
Do you consider contour augmentation for implant placement (simultaneous placement and GBR)?	yes	8 (57.1)	5 (71.4)	7 (70.0)	9 (69.2)	3 (60.0)	32 (65.3)	>0.05
	no	1 (7.1)	1 (14.3)	1 (10.0)	2 (15.4)	0 (0.0)	5 (10.2)	
	must for all cases	2 (14.3)	1 (14.3)	2 (10.0)	2 (15.4)	2 (40.0)	9 (18.4)	
	not much of clinical relevance	3 (21.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.1)	
When do you consider augmenting hard tissue?	when the available amount of bone is less than in the case of regular platforms	13 (92.9)	7 (100.0)	9 (90.0)	13 (100.0)	3 (60.0)	45 (91.8)	>0.05
	it doesn't matter, I have other options, like narrow platforms	1 (7.1)	0 (0.0)	1 (10.0)	0 (0.0)	2 (40.0)	4 (8.2)	
In case of deficiency, do you perform the hard tissue augmentation procedures by yourself?	yes	4 (28.6)	7 (100.0)	10 (100.0)	7 (53.8)	4 (80.0)	32 (65.3)	<0.001*
	no	10 (71.4)	0 (0.0)	0 (0.0)	6 (46.2)	1 (20.0)	17 (34.7)	
Which of the following techniques of ridge augmentation do you perform?	GBR	4 (100.0)	4 (57.1)	4 (40.0)	5 (71.4)	3 (75.0)	20 (62.5)	>0.05
	ridge split technique	0 (0.0)	0 (0.0)	2 (20.0)	0 (0.0)	0 (0.0)	2 (6.3)	
	autogenous block grafts	0 (0.0)	1 (14.3)	4 (40.0)	1 (14.3)	0 (0.0)	6 (18.8)	
	ridge expansion technique	0 (0.0)	1 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.1)	
	depends on the type of bone deficiency	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	1 (3.1)	
	all	0 (0.0)	1 (14.3)	0 (0.0)	1 (14.3)	0 (0.0)	2 (6.3)	

Data presented as *n* (%). GBR – guided bone regeneration; CBCT – cone-beam computed tomography; * statistically significant.

Discussion

Today, implant-supported prostheses have become a better treatment option for replacing missing natural teeth than FPD.[14] The knowledge regarding the anatomy, evaluation and management of soft and hard tissues is a prerequisite before implant placement, as it plays a vital role in the long-term success of implants.[15] In the current study, the quality and quantity of the bone at the implant site were a significant consideration for selecting the type of implant. However, the patient's financial capability was also a determining factor for 20.4% of the study participants.

Implants are classified as bone-level and tissue-level. Bone-level implants, in turn, can be classified into platform-switched (internal hex or conical connections) and

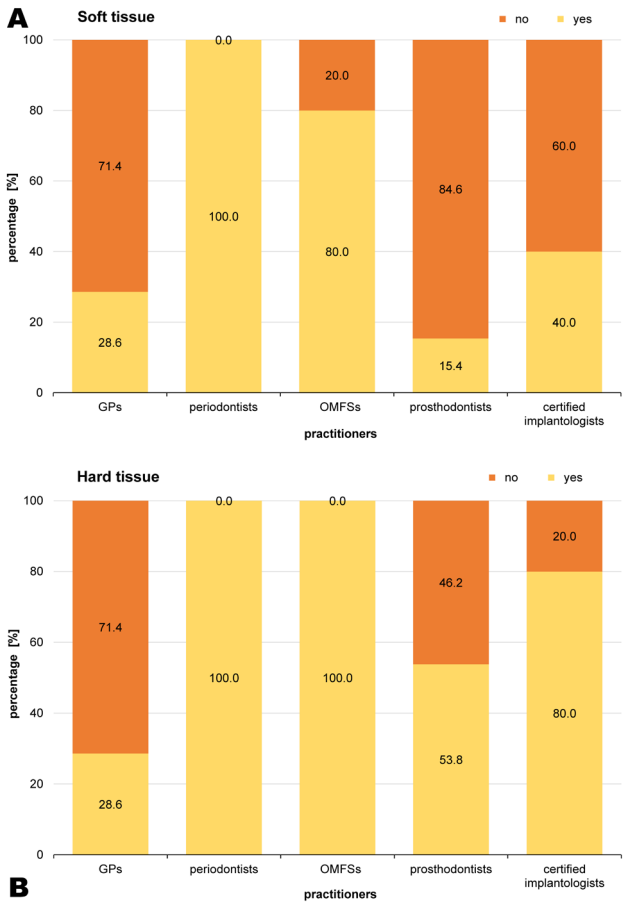

Fig. 3. In case of deficiency, do you perform augmentation by yourself? A – soft tissue; B – hard tissue.

platform-matched based on the type of implant–abutment connection.[8,16] A series of studies have demonstrated that platform-switched implants produce better crestal remodeling than platform-matched implants (0.5 mm vs. 2 mm of bone loss around the crest).[17–19] The majority of the study participants (67.3%) used platform-switched implants, while 57.1% of the GPs preferred platform-matched implants. Platform-switched implants have been introduced more recently than platform-matched implants.[20] Specialists, such as periodontists and prosthodontists, are well aware of the benefits of platform-switched implants, which was reflected in their selection of the implant system based on the quality and quantity of the available bone. The GPs lacked the knowledge about the latest innovations, as many of them had not attended any CDE programs in the last 2 years. In addition, platform-matched implants are comparatively cheaper than platform-switched implants, which could have influenced their choice in clinical practice (Table 1).

The soft tissue biotype has a vital role in preventing long-term peri-implant complications.[10] Most participants (93.9%) deemed the soft tissue biotype to influence the long-term treatment outcome, but only 67.4% considered it in all scenarios. Currently, various methods are available for evaluating the soft tissue biotype, such as probe visibility through the sulcus,[21] penetration with an endodontic file, ultrasonography (USG), and CBCT.[22] However, penetration with an endodontic file at the site gives a better estimation of the biotype, while probe transparency is also considered to give better results due to its minimal invasiveness.[23] Nevertheless, in the current study, most participants used either penetration with a probe at the implant site or the probe transparency method on the adjacent tooth to determine the soft tissue biotype.

The WKG has a critical role in the long-term maintenance of implants, especially if the patient is not compliant with oral hygiene.[10] In the current study, 67.3% of the respondents, including all periodontists, considered WKG before implant placement. There are various methods available to measure WKG, including the visual method, the histochemical method and using a probe to measure the keratinized gingiva from the mucogingival junction to the gingival margin,[24] with 81.8% of the study participants using the latter. All OMFSs and prosthodontists, and the majority of the periodontists utilized this method for evaluating the keratinized gingiva. However, less than half of the participants (46.9%) performed soft tissue augmentation. Periodontists and most OMFSs performed such procedures by themselves, while most GPs and prosthodontists called for consultants. Generally, tissue with a thick biotype has more connective tissue components than epithelial components as compared to a thinner biotype.[25] Hence, connective tissue is the gold standard in terms of biotype-switching.[26] Of all study participants, 42.9% preferred connective tissue as a biotype-switching material, while others preferred FGG and soft tissue substitutes. Meanwhile, 30.6% perceived SCTG to be the best material for increasing WKG, and 26.5% perceived FGG to be optimal, including as many as 71.4 of the periodontists. Nevertheless, current evidence suggests that autogenous grafts (SCTG and FGG) increase WKG,[26] though they have disadvantages, such as limited availability, postoperative pain and second-site morbidity. Hence, soft tissue substitutes, like xenogenic collagen matrices and acellular dermal matrices, have been used to replace autogenous grafts.[27] Also, pre-hydrated membranes that simulate the natural environment perform better than non-hydrated membranes.[28] However, the latest evidence indicates similar patient-reported outcomes (the endpoint of soft tissue grafting) with autografts and soft tissue substitutes.[29]

The bone width and length should be appropriately assessed before implant placement. Adequate bone width should be present to place regular platform implants in the restorative-driven position.[11] In the current study, 91.8% of the participants evaluated the bone width before implant placement, but only 77.6% evaluated it while considering the future prostheses, i.e., restoration-driven implant placement, among them 92.3% of the prosthodontists. The bone width and length can be evaluated using many invasive and non-invasive methods. Though direct

evaluation following flap opening is the gold standard, it is not feasible to plan surgery in the prosthodontic-driven implant position with the use of this method. Hence, CBCT is a better non-invasive method for estimating the bone width and length. Two out of 3 participants in the study utilized CBCT to evaluate the bone width, while 4 out of 5 used CBCT to evaluate the bone length. One striking finding in this aspect is that all of the prosthodontists employed CBCT, with or without other methods. In cases of inadequate width and length, bone narrow-platform implants or short implants are generally used to compensate. However, narrow-platform implants underperform as compared to regular-platform implants due to the reduced bone-to-implant contact surface,[30] and the evidence regarding short implants is still debatable.[31]

Another critical aspect for the long-term success of implants is the thickness of the buccal bone, with data showing that a minimum of 1.5–2 mm is required.[11] The buccal bone thickness of the anterior maxilla is usually less than 2 mm.[32–34] Hence, contour augmentation is generally indicated in most cases with the buccal bone thickness of less than 1.5 mm.[35] The facial bone thickness was evaluated by 83.7% of the study population, while contour augmentation with simultaneous implant placement was considered by 65.3% of the participants, but only 18.4% of the practitioners performed it in all cases. All periodontists and OMFSs performed hard tissue augmentation, which is likely due to the surgical training they seek during their postgraduate residency. Meanwhile, 62.5% of the study participants were familiar with GBR. The current study results suggest that implant practitioners give more attention to hard tissue than soft tissue. However, soft tissue has a decisive role in long-term maintenance. Since all study participants place implants, but not all perform augmentation, it is essential for everyone to have sound knowledge about the influence of soft tissue on diagnosis and management.

Our findings could be used to identify knowledge gaps in dental practitioners with different educational backgrounds. Most of the study participants had good knowledge regarding soft and hard tissues around implants. However, they lack awareness and opinions with regard to the abovementioned treatment, except for periodontists and oral surgeons. Specialist-specific CDE or CME programs should be conducted more often, and frequent reviews of knowledge and awareness should be performed. Implant dentistry is an interdisciplinary and rapidly developing science: it is the duty of every dental practitioner placing implants to learn about the latest advances and evidence, and learning should be considered a continuous process.

Limitations

As far as the authors are aware, this is the first study to assess implant practitioners' knowledge, awareness and opinions regarding soft and hard tissue considerations at a single implant site. However, the study has inherent limitations, such as a small sample size in a localized area, comparatively inexperienced practitioners, and questions limited to the clinical aspects of soft and hard tissue considerations at the implant site. Future studies in different regions, with a broader range of questions would help to understand the discrepancies between the evidence provided in the literature and applications in clinical practice.

Conclusions

The current study identified various knowledge gaps related to the different backgrounds of implant practitioners. Our findings suggest that education and knowledge play a key role in determining the attitude toward treatment planning and the subsequent material selection. Comprehensive knowledge of soft and hard tissues is essential, as all study participants practice implant dentistry.

Ethics approval and consent to participate

The research was approved by the institutional ethics committee at the Indian Dental Association Dakshina Kannada (IDA-DK) (Ref. No. ETHICS/ABSMIDS/135/2021). 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

Evaluation of soft tissue and crestal bone changes around non-submerged implants with and without a platelet-rich fibrin membrane: A randomized controlled clinical trial

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Abstract

Background. Platelet-rich fibrin (PRF) membranes are known to enhance wound healing after periodontal surgeries and dental implant procedures.

Objectives. The aim of the present study was to examine the effect of PRF on soft tissue healing and the crestal bone level (CBL) around non-submerged dental implants.

Material and methods. A total of 40 patients, aged 20–60 years, with partially edentulous posterior mandibular sites were divided into 2 groups of 20 patients each: group I received non-submerged implants with a PRF membrane; and group II was treated with non-submerged implants alone. The examined parameters included the modified plaque index (mPI), the gingival index (GI), the width of keratinized tissue (WKT), the thickness of keratinized tissue (TKT), and CBL, assessed using digital intraoral periapical radiography (IOPA). All parameters were measured at baseline (immediately post-op), and at 3-month and 6-month follow-ups.

Results. In comparison with baseline, statistically significant increases in WKT and TKT were observed in both groups at 3 and 6 months post-op ($p < 0.05$). Also, significant gains were noted in group I vs. group II ($p < 0.05$). The CBL increased significantly in both groups at 3 and 6 months post-op ($p < 0.05$), with no remarkable differences from 3 to 6 months. A decreased CBL was observed in group I vs. group II at the 3- and 6-month intervals ($p < 0.05$).

Conclusions. The PRF membrane enhances peri-implant tissue wound healing, with gains in soft tissue width and thickness around non-submerged implants.

Keywords: dental implants, wound healing, platelet-rich fibrin membrane

Introduction

Replacing missing teeth in edentulous sites with implants is the preferred and pragmatic alternative to fixed partial dentures and complete dentures.[1] With an impressive success rate of 90–100% for implant survival and prosthetic outcomes, a single-tooth endosseous implant is the most widely used form of dental implant.[2]

The initial concept for implant installation was a two-stage surgical intervention, which comprised a phase of submerged healing to achieve optimal osseointegration and implant longevity.[3] However, studies by Ferrigno et al. (2002) and Romeo et al. (2004) showed that satisfactory osseointegration could be achieved, with success rates being comparable to submerged implants, by using a single-stage procedure that does not require the implant to be submerged.[acc.4] The replacement procedure using a non-submerged implant involves the placement of the implant and the transmucosal abutment during the same surgery; the abutment, while uncovered in the oral cavity, heals and is gradually loaded to ensure osseointegration.[5] Non-submerged implants allow early implant loading, avoiding a second surgery and simplified prosthetic handling, with no subgingival micro-gaps between the implant and the abutment.[5,6]

Over time, autologous platelet derivatives, such as platelet-rich fibrin (PRF) have been used to augment soft tissue healing and rapid osseous regeneration by enhancing the chemotaxis, angiogenesis, mitosis, and proliferation of potent stem cells.[7,8] The physiological architecture of PRF, a second-generation platelet derivative, comprises a fibrin matrix, and platelets, leukocytes, cytokines, and growth factors. Therefore, PRF is used as an autologous barrier membrane in a variety of clinical procedures, such as extraction socket healing and sinus lifting, in implant dentistry, and for treating intraosseous periodontal defects and gingival recessions; it is also a novel treatment option for various mucogingival surgeries.[9,10]

A prerequisite for the longevity of an osseointegrated implant is its mechanical stability, which, in turn, depends on the quality and quantity of the surrounding soft tissue and bone.[11] The effect of PRF on the existing osseous structure and gingival architecture around non-submerged implants is not well documented.

According to our null hypothesis, there is no additional benefit with the PRF usage regarding the improvement of the soft and hard tissue parameters. Therefore, the present study aimed to examine and compare how PRF affects soft tissue healing and the crestal bone level (CBL) around a non-submerged implant.

Material and methods

Sample size calculation

For the sample size estimation, the G*Power software, v. 3.1.0 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower), was used. When the study power was set at 80%, with an alpha error of 5% and an effect size of 0.25%, the estimated sample size was 18. A sample of 20 was used to provide more robust results. As such, 40 implants (20 in each group) were placed.

Study design

This double-blind prospective randomized controlled clinical trial was conducted between May 2018 and April 2019. Patients with partially edentulous posterior mandibular sites were selected from the outpatient clinic of the Department of Periodontology, Sibar Institute of Dental Sciences, Guntur, India. All potential participants received information about the study design, and written informed consent was obtained from those willing to participate. A total of 40 patients (23 males and 17 females) were included in the study. The institutional ethics committee approved the study (Ref. No. 111/IEC SIBAR/Lr/17), and it was registered with the Clinical Trials Registry – India (CTRI) (No. 2018/05/013713).

Randomization and allocation

The 40 patients were randomly divided into 2 groups of 20 participants: group I received non-submerged implants (cylindrical implants with a parallel body and a core design) with a PRF membrane; and group II received non-submerged implants alone. Randomization was carried out by an independent researcher (C.A.), who was unaware of the study design, by using a computer-generated code at a 1:1 ratio. Allocation was concealed in opaque envelopes labeled with patient study numbers, which were opened before the surgical procedure. The study followed the CONSORT (Consolidated Standards of Reporting Trials) guidelines, and the CONSORT flow diagram is shown in Fig. 1.

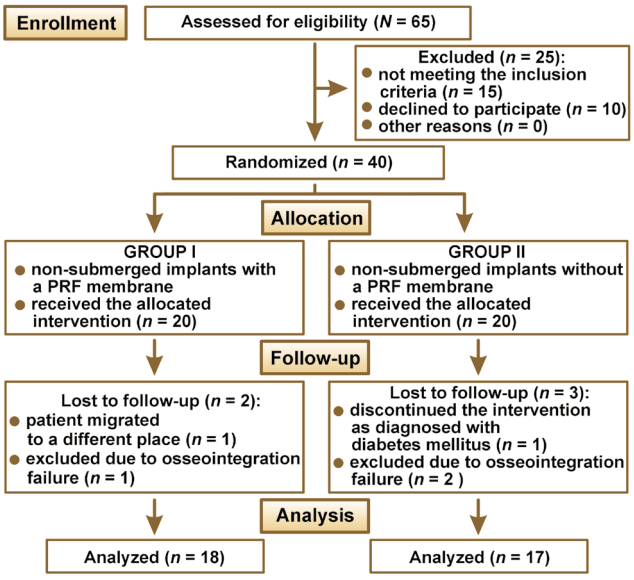

Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) flowchart
PRF – platelet-rich fibrin.

Inclusion and exclusion criteria

Patients aged 20–60 years with a partially edentulous posterior mandibular site containing sufficient bone to allow implant installation, without the need for a ridge augmentation procedure were included in the study.

Systemically compromised patients or those taking any medications/drugs that may affect the treatment outcome, patients with a history of osteoporosis, patients with a history of tobacco use, those unable to perform routine oral hygiene procedures, and those with a history of parafunctional habits, such as bruxism, were excluded from the study.

Clinical and radiographic parameters

The outcome measures consisted of clinical parameters, including the modified plaque index (mPI),[12] the gingival index (GI),[13] the width of keratinized tissue (WKT),[14] and the thickness of keratinized tissue (TKT).[15] Radiographic parameters included CBL, measured using digital intraoral periapical radiography (IOPA).[16] An independent examiner (R.D.), who was unaware of the study design, measured all clinical parameters. The WKT was measured using a calibrated manual periodontal probe between the gingival margin and the mucogingival junction (MGJ).[14] The TKT was measured after anesthetizing the area using a 15-millimeter endodontic reamer by gently inserting it at the midpoint, 2 mm apical to the gingival margin on the facial aspect (perpendicular to the tooth surface), until the underlying hard structures were contacted, and the distance was measured with a University of North Carolina 15 (UNC 15) periodontal probe.[15] Digital IOPA radiographs were taken using a 1 × 1 mm grid to measure CBL from the implant crest to the most apical point of the interproximal crestal bone on both the mesial (M) and distal (D) surfaces; an average value for these measurements was computed: (M + D)/2.[16,17]

At their index visit, patients received full-mouth scaling, root planing and oral hygiene instructions, and they were recalled after 2 weeks for implant placement.

Preparation of platelet-rich fibrin

Before the surgical procedure, a 10-cc blood sample was collected from the patient's antecubital vein, transferred to a test tube without an anticoagulant, and instantly centrifuged at 2,700 rpm for 12 min.[18] We removed the fibrin clot that developed in the mid-portion of the tube and compressed it in the PRF box to obtain a PRF membrane.

Surgical procedure

Under adequate local anesthesia, a sulcular mid-crestal incision was made extending to the adjacent tooth (Fig. 2A), and a full-thickness mucoperiosteal flap was reflected on both the buccal and lingual sides (Fig. 2B). The initial bone marking used a surgical template. For placing a tapered cylindrical endosseous implant, the osteotomy site was prepared using a series of drills, starting with a 2-millimeter pilot drill, with a speed ranging from 500 to 1,200 rpm under copious irrigation until the desired length was established (Fig. 2C). Afterward, the implant was installed, and primary stability was assessed using the resonance frequency analysis (RFA) device (Osstell, Gothenburg, Sweden) (Fig. 2D). Next, we placed the healing abutment on the implant with the use of a torque control device.

In group I, the PRF membrane was placed along with the healing abutment, using the poncho technique to cover the implant (Fig. 2E).[19] The healing abutment was tight-

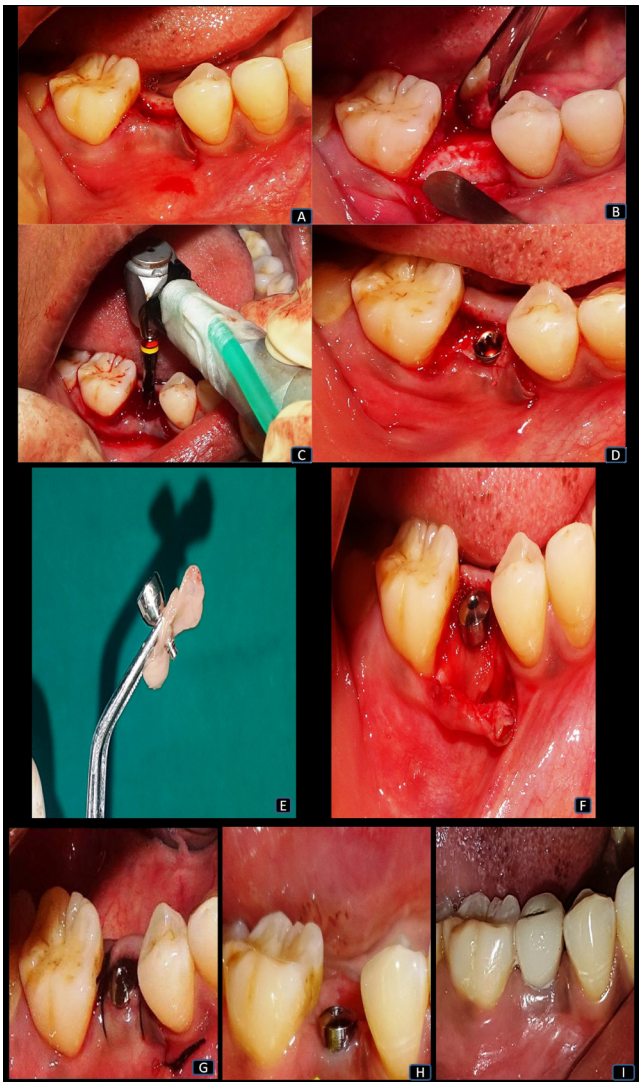

Fig. 2. Surgical procedure

A – mid-crestal incision and a releasing incision given at region 45; B – full-thickness mucoperiosteal flaps elevated both buccally and palatally; C – drilling of the osteotomy site with drills under saline irrigation; D – implant placed into the osteotomy site; E – healing abutment with the PRF membrane (the poncho technique); F – healing abutment placed to the implant with the PRF membrane; G – flaps approximated and sutures placed; H – abutment at 3 months post-op after healing; I – cement-retained porcelain-fused-to-metal ceramic crown placed at 3 months post-op.

ened to the PRF membrane so that the PRF membrane covered the implant and the alveolar crestal interface (Fig. 2F). Subsequently, the flap margins were approximated and sutured in both groups, using non-absorbable 3-0 black silk sutures (Fig. 2G).

Postoperatively, amoxicillin (500 mg thrice daily) and analgesics (100 mg aceclofenac, 500 mg paracetamol and 15 mg serratiopeptidase twice daily) were prescribed for 5 days, along with 0.2% chlorhexidine gluconate twice daily for 15 days. Postoperative instructions were given to all patients, and they were recalled after 1 week for suture removal. All surgical procedures and postoperative care were carried out by the primary investigator (R.N.S.C.).

At 3 months post-op, the healing cap was replaced with a prosthetic abutment (Fig. 2H). The restoration was provided with a cement-retained porcelain-fused-to-metal ceramic crown (Fig. 2I). All outcome measures were evaluated immediately post-op (baseline), and at 3 months and 6 months post-op for both groups. Figure 3 shows the digital IOPA radiographs for both groups.

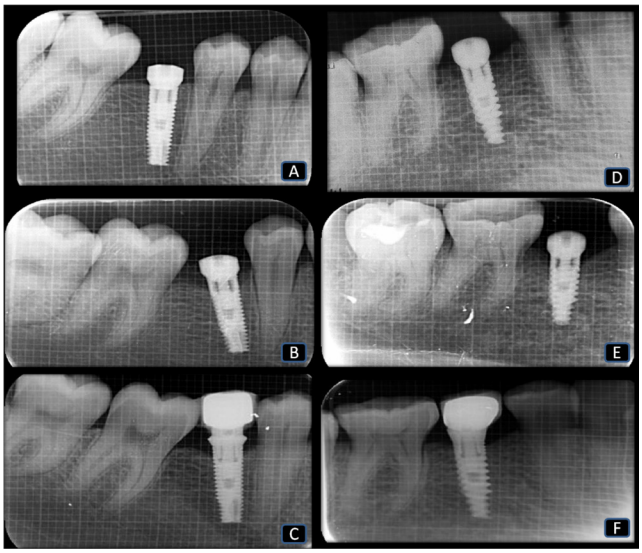

Fig. 3. Digital intraoral periapical (IOPA) radiographs taken at baseline (immediately post-op), and 3 months and 6 months post-op in group I (A,B,C, respectively) and group II (D,E,F, respectively)

Statistical analysis

Statistical analysis employed IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA), and was performed by an independent statistician who was blinded to the study groups. After calculating the mean (*M*) and standard deviation (*SD*) values, the 2 groups were compared with regard to mPI, GI, WKT, TKT, and CBL at baseline, 3 months and 6 months, using the Mann–Whitney *U* test. Within-group analysis used the Wilcoxon matched-pairs test. A *p*-value <0.05 indicated statistical significance.

Results

Out of the 40 implants, 3 (1 in group I and 2 in group II) failed to osseointegrate, and 2 patients (1 in each group) did not attend the follow-up examination. Therefore, the analysis was carried out for 35 patients: group I (n = 18; 10 males and 8 females; mean age: 37.11 ±10.43 years); and group II (n = 17; 8 males and 9 females; mean age: 35.88 ±10.76 years) (Tables 1,2).

Table 1. Comparison of the groups by age

Age groups	Group I	Group II	Total
M ±SD [years]	37.11 ±10.43	35.88 ±10.76	36.51 ±10.45
21–30 years	5 (27.78)	6 (35.29)	11 (31.43)
31–40 years	9 (50.00)	5 (29.41)	14 (40.00)
≥41 years	4 (22.22)	6 (35.29)	10 (28.57)
Total	18 (100.00)	17 (100.00)	35 (100.00)

Data presented as number (percentage) (n (%)). *M* – mean; *SD* – standard deviation; $p = 0.448$; $\chi^2 = 1.6071$.

Table 2. Comparison of the groups by gender

Gender	Group I	Group II	Total
Male	10 (55.56)	8 (47.06)	18 (51.43)
Female	8 (44.44)	9 (52.94)	17 (48.57)
Total	18 (100.00)	17 (100.00)	35 (100.00)

Data presented as n (%). $p = 0.615$; $\chi^2 = 0.2531$.

Clinical parameters

The mean mPI and GI scores were statistically similar for both groups at the 3- and 6-month time points, and between 3 months and 6 months ($p > 0.05$). Within the groups, the mean mPI and GI values did not show any significant differences when compared between 3 months and 6 months (group I: $p = 0.081$, $p = 0.592$; group II: $p = 0.063$, $p = 0.463$, respectively) (Table 3).

The mean WKT values for groups I and II showed statistically significant changes between baseline and 3 months ($p = 0.003$ and $p = 0.027$, respectively), between baseline and 6 months ($p = 0.000$ and $p = 0.001$, respectively), and between 3 months and 6 months ($p = 0.002$ and $p = 0.003$, respectively). Besides the statistically significant difference from baseline, a gain in WKT was also observed in group I vs. group II at 6 months post-op ($p < 0.05$) (Table 3).

Statistically significant increases in TKT were achieved for groups I and II between baseline and 3 months ($p = 0.001$ and $p = 0.016$, respectively), and between baseline and 6 months ($p = 0.000$ and $p = 0.007$, respectively). Meanwhile, a significant difference was observed in group I between 3 months and 6 months ($p = 0.018$), unlike in group II ($p = 0.970$). When comparing the mean TKT scores between the 2 groups, significant gains were observed in group I vs. group II at 3 and 6 months post-op ($p < 0.05$) (Table 3).

Table 3. Comparison of the clinical parameters between the groups at different time intervals

Parameter	Time interval	Group I $n = 18$	Group II $n = 17$	*p*-value
mPI	3 months	1.09 ±0.50	1.21 ±0.77	0.591
	6 months	0.94 ±0.24	1.00 ±0.00	0.779
	3–6 months	0.15 ±0.51	0.21 ±0.77	0.809
GI	3 months	0.42 ±0.35	0.37 ±0.28	0.754
	6 months	0.38 ±0.12	0.32 ±0.16	0.621
	3–6 months	0.04 ±0.30	0.05 ±0.21	0.438
WKT [mm]	baseline	3.03 ±0.27	3.06 ±0.50	0.817
	3 months	3.67 ±0.66	3.24 ±0.47	0.062
	6 months	4.28 ±0.60	3.65 ±0.39	0.003*
	baseline–3 months	0.64 ±0.66	0.18 ±0.25	0.036*
	baseline–6 months	1.25 ±0.67	0.59 ±0.36	0.003*
	3–6 months	0.61 ±0.47	0.41 ±0.36	0.187
TKT [mm]	baseline	2.00 ±0.30	1.41 ±0.48	0.002*
	3 months	2.64 ±0.54	1.88 ±0.63	0.003*
	6 months	2.97 ±0.36	2.00 ±0.53	0.000*
	baseline–3 months	0.64 ±0.45	0.47 ±0.57	0.438
	baseline–6 months	0.97 ±0.12	0.59 ±0.57	0.031*
	3–6 months	0.33 ±0.45	0.12 ±0.33	0.192

Data presented as *M* ±*SD*. mPI – modified plaque index; GI – gingival index; WKT – width of keratinized tissue; TKT – thickness of keratinized tissue; * statistically significant.

Radiographic parameters

The CBL showed a statistically significant increase in both groups independently at 3 and 6 months post-op ($p < 0.05$), while the difference was not significant for the time interval 3 to 6 months. The mean difference in CBL from 3 to 6 months within group I was 0.43 ±0.53 mm, while it was 0.54 ±0.48 mm for group II. A statistically significant decrease in CBL was observed in group I vs. group II at the 3- and 6-month time points ($p < 0.05$) (Tables 4,5).

Discussion

To the best of our knowledge, none of the available studies on the use of PRF in various surgical procedures have considered its potential significance in early soft tissue healing or evaluated CBL around non-submerged implants. The current comparative study examined the clinical and radiological effects of PRF on the soft and hard tissues surrounding non-submerged implants. We placed 40 implants in posterior mandibular edentulous sites based on the quality and quantity of the available bone, initially evaluated using cone-beam computed tomography (CBCT) for diagnostic purposes.[20] Of these implants, 3 failed to osseointegrate, and 2 patients were lost to follow-up. As such, results from 35 non-submerged implants were analyzed, and functional soft tissue integration and patient satisfaction were obtained.

He et al. described advancing age to be associated with decreased bone density, which further affects implant osseointegration.[21] Therefore, we included patients aged between 20 and 60 years (mean age: approx. 36 years).

Numerous growth and differentiation factors, such as bone morphogenic proteins and lactoferrin, enhance bone regeneration. Also, vitamin D plays a vital role in osseointegration by maintaining calcium and phosphorus homeostasis during bone metabolism. Optimal levels of 25-hydroxycholecalciferol significantly increase the bone level at the implant site during osseointegration, according to Kwiatek et al.[22] Therefore, evaluating vitamin D levels in the current study could have improved the treatment outcomes.

Plaque development around non-submerged implants is an important etiological factor for peri-implant disease and gingival tissue inflammation. The present study found no significant increase in the mPI and GI scores around the implants in either group. These findings can be attributed to the repeated reinforcement

Table 4. Comparison of the crestal bone level (CBL) scores [mm] between the groups at 3- and 6-month time points (Wilcoxon matched-pairs test)

Group	Time point	*M* ±*SD*	*MD*	Effect [%]	Paired *t*	*p*-value
Group I	3 months	1.10 ±0.80	0.43 ±0.53	–39.24	2.6889	0.0072*
	6 months	1.53 ±0.38				
Group II	3 months	1.34 ±0.41	0.54 ±0.48	–40.66	3.4078	0.0007*
	6 months	1.88 ±0.64				

MD – mean difference; * statistically significant.

Table 5. Comparison of the crestal bone level (CBL) scores [mm] between the groups at 3- and 6-month time points (Mann–Whitney *U* test)

Time point	Group I		Group II		*U*-value	*Z*-value	*p*-value
	M ±*SD*	mean rank	*M* ±*SD*	mean rank			
3 months	1.10 ±0.80	14.08	1.34 ±0.41	22.15	82.50	–2.3268	0.0200*
6 months	1.53 ±0.38	14.22	1.88 ±0.64	22.00	85.00	–2.2443	0.0248*
3–6 months	0.43 ±0.53	18.11	0.54 ±0.48	17.88	151.00	–0.0660	0.9474

* statistically significant.

of oral hygiene measures given to the patient, as well as the patient's compliance with maintaining good oral hygiene.[23] On comparing the within-group change in scores, group I showed reduced mPI and GI scores, indicating that the PRF membrane, when placed around implants, enhances angiogenesis and maintains tissue integrity.

We observed a considerable rise in the WKT and TKT values in both groups, which may be due to the functional soft tissue integration and adaptation achieved from the immediate placement of the healing abutment, unlike in the case of the two-stage implant procedure, where it is often sacrificed for the reopening surgery to gain access to the implant fixture and place the abutment.[24,25] These findings concur with previous studies by Temmerman et al.[26] and Linkevicius et al.,[27] who reported better gains in WKT and TKT with the placement of a PRF membrane around the implant. Furthermore, the increases in WKT and TKT may have also contributed to the lower mPI and GI scores, since an adequate zone of keratinized mucosa is crucial for preventing the development of plaque and the subsequent peri-implantitis, and for preserving the health of the surrounding tissues.[26–28]

A statistically significant, although minimal, decrease in CBL was observed in group I at 3 and 6 months post-op as compared to group II, which may be explained by the concept of 'natural bone regeneration' given by Simonpieri et al.[29] They suggested that the bone volume and gingival tissues regenerated through the PRF membrane due to the presence of multiple growth factors in its structure, such as platelet-derived growth factors and transforming growth factors.[29] The factors are engaged in the osteoblastic activity and tissue regeneration by stimulating the osteoblastic response, with stable effects that improve bone formation.[30,31]

We preferred using digital IOPA radiographs over CBCT following implant insertion due to the possibility of obtaining radiographic image artifacts, especially through metal restorations, like implants.[32] It is accepted that employing CBCT after implant placement should be limited to complex surgical procedures for postoperative complications, like iatrogenic neurovascular trauma or implant recovery.[33]

The current study demonstrated that, when applied locally during a dental replacement procedure, PRF membranes facilitate the healing of the soft tissue surrounding the implant and reduce crestal bone resorption.

Limitations

The study was limited by a small sample size, a short follow-up period, and the evaluation of CBL in only the mesial and distal sides, using two-dimensional (2D) radiographs. Also, the radiographic projections were not standardized, and vitamin D levels were not measured before implant installation. However, there is a need for more meticulously designed longitudinal studies with larger samples that would account for the different biotypes and bone volumes (D1, D2, D3, and D4) to understand the influence of PRF on the adjacent tissues.

Conclusions

Our study shows that the placement of a PRF membrane with a non-submerged implant enhances peri-implant tissue wound healing, with gains in soft tissue width and thickness around the implant.

Future perspectives

The focus of the current study was to evaluate the effects of PRF membranes on the soft and hard tissue parameters around dental implants. However, the number of PRF membranes necessary to obtain soft and hard tissue benefits is yet to be determined. Even though it is possible to use PRF in almost every procedure in implant dentistry, its potential long-term clinical benefits have not been established. Nonetheless, the ease of manipulation, its autologous nature and the release of growth factors support PRF utilization in implant dentistry. Thus, the present study is a stepping stone for future randomized controlled trials.

Ethics approval and consent to participate

The study was approved by the institutional ethics committee at the Sibar Institute of Dental Sciences, Guntur, India (Ref. No. 111/IEC SIBAR/Lr/17), and it was registered with the Clinical Trials Registry – India (CTRI) (No. 2018/05/013713). 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

Antibacterial and anti-inflammatory effects of a novel herb-mediated nanocomposite mouthwash in plaque-induced gingivitis: A randomized controlled trial

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Abstract

Background. Gingivitis is frequently painless, rarely causes spontaneous bleeding and is manifested by minor clinical changes. Therefore, most patients are unaware of the disease or do not seek treatment, as it is asymptomatic. Several methods for removing microbial plaque have been proposed, including mechanical and chemical ones. Amla or Indian gooseberry is a medicinal herb; its secondary metabolites, such as phenolic acid, flavonoids and terpenoids, can be used to preferentially reduce metal ions and form nanoparticles (NPs). Green synthesis with the use of the amla seed extract is a unique approach for the production of graphene oxide (GO)-silver (Ag) nanocomposite mouthwash.

Objectives. The aim of the present study was to prepare an amla seed-mediated GO-Ag nanocomposite mouthwash, and to assess its antibacterial and anti-inflammatory efficacy in plaque-induced gingivitis.

Material and methods. The present double-blind randomized controlled trial was conducted among 30 gingivitis patients. The patients were randomly allocated into 2 groups based on the intervention: group A ($n = 15$; nanocomposite mouthwash); and group B – control ($n = 15$; 0.2% chlorhexidine (CHX) mouthwash). Clinical parameters, including the plaque index (PI), the gingival index (GI), a microbiological parameter – colony forming units (CFUs), and a biochemical parameter – the C-reactive protein (CRP) level in gingival crevicular fluid (GCF), were assessed at baseline and at 15 days.

Results. The study results showed statistically significant differences in the mean PI and GI scores, and the CRP levels in the post-intervention period as compared to baseline in both groups. After the intervention period of 15 days, there were statistically significant differences between the 2 study groups in terms of mean PI and GI scores, and CRP levels.

Conclusions. The amla seed-mediated GO-Ag nanocomposite mouthwash efficiently reduced plaque, gingival inflammation and CFUs among patients with plaque-induced gingivitis, but was not equivalent to the CHX mouthwash.

Keywords: graphene oxide, nanoparticle, silver

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Introduction

Dental caries and gingivitis have been identified as the most common oral diseases across the globe. Gingivitis is an inflammatory condition characterized by gingival redness, edema and a lack of periodontal attachment loss, and is typically caused by dental biofilm accumulation.[1] Furthermore, it is often painless, rarely causes spontaneous bleeding and is manifested by minor clinical changes. Therefore, most patients are unaware of the disease or do not seek treatment, as it is asymptomatic. Gingivitis can be plaque-induced or non-plaque-induced. However, in children and adults, plaque-induced gingivitis is considered the most prevalent form of periodontal disease. Plaque-induced gingivitis is defined as an inflammatory response of gingival tissues, resulting from bacterial plaque accumulation at and below the gingival margin.[2] If left untreated, gingivitis can proceed to periodontitis, which involves the loss of periodontal attachment and the supporting alveolar bone, eventually leading to tooth loss, which is closely related to a negative impact on quality of life.[3]

In comparison with periodontitis, plaque-induced gingivitis is unique in that the tissue changes are completely reversible if biofilm accumulation is controlled. Several methods for removing microbial plaque have been proposed, including mechanical and chemical ones. According to data, mechanical plaque control does not appear to be 100% effective in certain conditions. As a result, chemical plaque control has received much attention. Chemical plaque control agents have antimicrobial, anti-plaque or anti-gingivitis properties. Chlorhexidine gluconate (CHX) and essential oils are the active chemical components in widely prescribed mouthwashes. Chlorhexidine gluconate is still considered the gold standard agent among various commercially available mouthwashes.[4] Depending on the concentration, CHX acts as a bacteriostatic or bactericidal agent. The long-term use of CHX-containing drugs, despite their effectiveness in lowering the microorganism levels in the oral cavity, is associated with local side effects, such as impaired taste, tooth staining, increased supragingival calculus production, and intermittent inflammation and desquamation of mucous membranes.[5]

Herbal compounds have been included as ingredients in oral care products for a long time, especially in South Asian nations. Sanguinary, clove, propolis, miswak, neem, and charcoal are the most common herbal compounds included in toothpastes and mouthrinses.[6] Natural products derived from medicinal plants have proven a rich supply of physiologically active molecules, with many serving as the foundation for the development of new pharmaceutical chemicals. In Indian medicine, *Phyllanthus emblica* or *Emblica officinalis*, commonly called amla or Indian gooseberry, is a valuable medicinal plant. It contains vitamin C, polyphenols, flavonols and tannins, and has antibacterial, antifungal, antiviral, antioxidant, and wound healing properties and other pharmacological effects.[7] Several clinical trials on amla have found that it efficiently inhibits the formation of bacterial plaque, preventing the progression of gingivitis and periodontitis.[8]

The elimination of the biofilm matrix and resident bacteria by using nanoparticles (NPs) shows a lot of promise.[9] Nanoparticles have a diameter of less than 100 nm, and the use of their unique properties to resist infection has expanded dramatically in the last decade. The ability of NPs to inhibit the formation of biofilms within the oral cavity, as a result of their biocidal, anti-adhesive and delivery capacities, is currently being closely examined. Among all NPs, silver nanoparticles (AgNPs) have significant antibacterial properties.[10–14] Bacteria are less prone to develop resistance to AgNPs than to conventional antibiotics. As a result, combining graphene oxide (GO) and AgNPs to build a nanocomposite makes it a superior antibiotic to either component alone.[15] The GO–Ag bond is stable, has a high oxidative potential and is hydrophilic, which results in oxidative stress. Silver releases toxic components that have a bactericidal effect, whereas GO wraps around the bacterium.

Plant-mediated nanoparticle production has recently received much attention.[16] A number of plant products, such as extracts, are used to produce metal NPs, such as gold (Au), Ag, copper (Cu), and zinc (Zn) NPs.[17] Plant secondary metabolites, such as phenolic acid, flavonoids, terpenoids, and alkaloids, are abundant in crude extracts; they preferentially reduce metal ions and form NPs. Green synthesis with the use of the amla seed extract is a unique approach to the production of GO-AgNP composites.

As plaque control is the key factor for the prevention of disease progression from gingivitis to periodontitis, the formulation of a novel nanocomposite could be clinically beneficial. A preliminary study including the preparation and characterization of an amla seed-mediated GO-Ag nanocomposite found antibacterial activity against *Streptococcus mutans*, *Lactobacillus* and *Candida albicans*, using an in vitro cytotoxicity assay.[18] Therefore, the present study aimed to prepare an amla seed-mediated GO-Ag nanocomposite mouthwash, and to assess its antibacterial and anti-inflammatory efficacy in plaque-induced gingivitis.

Material and methods

Setting and design

The study was a double-blind randomized controlled trial carried out at Saveetha Dental College and Hospitals, Chennai, India. The study was designed in accordance with the Declaration of Helsinki. Ethical clearance was obtained from the institutional ethics committee (IEHC/PERIO-18/2022/35), and the study participants provided written informed consent before the intervention. The study followed the CONSORT (Consolidated

Standards of Reporting Trials) guidelines. The sample size was determined using the standard deviation (*SD*) values of a previous study[19] with the G*Power software, v. 3.0 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower), and was estimated to be 14, which was rounded to 15 for each group, and the total sample size was 30. Randomization used online random allocation software (RandomAlloc.exe., v. 1.0; https://random-allocation-software.software.informer.com/1.0), where the number of groups, the number of patients per group, and a number range were fed into the software to generate random numbers. Each patient was allotted a number, according to which they were allocated to either of the groups: group A (nanocomposite mouthwash); or group B – control (0.2% CHX mouthwash). The study design is depicted as a CONSORT flow diagram in Fig. 1. The patient and the clinician were blinded. To avoid bias, the mouthwashes were given to the subjects in an amber-colored bottle.

The inclusion criteria were as follows: plaque-induced gingivitis patients; age ranging from 18 to 30 years; with a minimum of 20 teeth; no other systemic conditions; participants with plaque index (PI) and gingival index (GI) scores ≥1 in 10% of the sites; and participants willing to comply with the appointment schedule.

The exclusion criteria were as follows: generalized or localized chronic periodontitis; pregnant or lactating women; participants with any systemic condition; participants with any allergy or infectious disease; participants receiving antibiotic therapy or any medication within the past 6 months; participants using any chemical mouthwash or any other oral hygiene aids; participants wearing an orthodontic appliance or a removable partial denture; and patients who refused to be a part of the study.

Preparation of the herbal mouthwash

Preparation of the amla seed extract

Fresh amla fruits were collected, washed thoroughly and cut to obtain seeds. The seeds were finely crushed into a powder. The powdered amla seeds weighing around 25 g were carefully cleaned in distilled water for 5 min before being heated for 15 min at 70°C in a 500-milliliter Erlenmeyer flask with 100 mL sterile distilled water, and filtered. The Ag ions and the GO ions were bio-reduced to NPs by using the filtrate.

Synthesis of amla seed-mediated GO-Ag NPs

The amla seed extract (10 mL) was added to a 1mM aqueous Ag nitrate solution (sample A), and 10 mL of the amla seed extract was added to a 1mM aqueous GO solution (sample B). Both samples were incubated in the dark for 24 h, after which ultraviolet-visible (UV-VIS) spectrophotometry was used to determine the maximum absorbance. To produce GO-Ag nanocomposite samples, an equal proportion of both samples (sample A and sample B) were combined together (sample C). All samples were then heat-dried to obtain the synthesized AgNPs, GONPs and a GO-Ag nanocomposite.

Preparation of the nanocomposite mouthwash

The main component were GO-Ag NPs, which were solubilized by using ethanol as a solvent (0.2%). The clove oil served as the flavoring agent, while sodium benzoate acted as a preservative. Other additives, including sucrose, sodium dodecyl phosphate and distilled water, were also added.

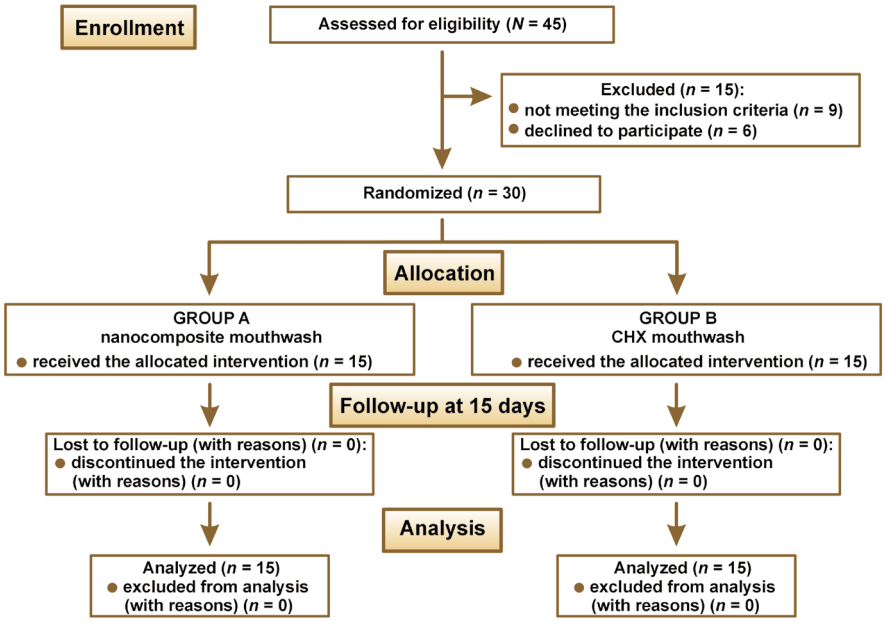

Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) flowchart
CHX – chlorhexidine.

Observational parameters

At baseline (day 0), oral prophylaxis was performed for all patients, and they were provided with the mouthwashes of their respective groups and received similar oral hygiene instructions. The patients were recalled 15 days after baseline. There were no reported side effects from the use of either the test or control solutions. At the baseline and at the revisit (after 15 days), the Silness–Loe PI and Loe–Silness GI were recorded. Gingival crevicular fluid (GCF) and subgingival plaque samples were collected at baseline and at the 15-day follow-up to assess the C-reactive protein (CRP) levels and colony-forming units (CFUs), respectively.

Collection and processing of gingival crevicular fluid

The crevicular site was dried and isolated with the use of a cotton roll. A standardized volume of GCF was collected by inserting a white color-coded calibrated 1–5-microliter microcapillary pipette from the test site, placing the tip of the pipette extra crevicular. The GCF was immediately placed into plastic vials containing phosphate-buffered saline (PBS) and frozen at 70°C. The CRP levels in the GCF samples were assessed with a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Calbiotech, Inc., El Cajon, USA), following the manufacturer's instructions.

Collection and processing of subgingival plaque

At each visit, subgingival plaque samples were taken for microbiological investigation. The sample was transferred to a 5-milliliter Eppendorf tube containing a thioglycolate transport medium. The samples were inoculated into a brain heart infusion (BHI) medium, using the streak culture technique. The plates were incubated at 37°C in an anaerobic jar with a gas-pak sachet. The CFUs were counted after 48 h.

Statistical analysis

The IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA), was used to perform statistical analysis. Intergroup analysis of the mean PI and GI scores, and CRP levels used the independent t test, whereas intragroup analysis at different time intervals employed Student's paired t test. With regard to CFUs, the Mann–Whitney U test was performed for intergroup analysis, and the Wilcoxon signed-rank test was carried out for intragroup analysis. The level of significance was set at $p < 0.05$.

Results

Table 1 shows the results of Student's paired t test and the independent t test for intragroup intergroup comparisons, respectively. There were statistically significant differences in the mean PI and GI scores, and CRP levels in the post-intervention period as compared to baseline in both groups ($p < 0.05$). Hence, it is evident that the nanocomposite mouthwash and the CHX mouthwash were equally potent in reducing the PI and GI scores, and the CRP levels. Intergroup comparisons showed no statistically significant differences between group A and group B at baseline for PI, GI and the CRP levels. However, after the intervention period of 15 days, there were statistically significant differences between the 2 study groups in terms of mean PI and GI scores, and CRP levels ($p < 0.05$).

Table 1. Comparisons of the mean plaque index (PI) and gingival index (GI) scores, and C-reactive protein (CRP) levels at different time intervals within each study group (intragroup comparison – Student's paired t test) and between the 2 study groups (intergroup comparison – the independent t test)

Parameter	Time interval	Group A	Group B	p-value
PI	baseline	2.92 ±0.12	2.76 ±0.46	0.180
	15 days	2.58 ±0.27	2.00 ±0.36	0.001*
	p-value	0.002*	0.001*	–
GI	baseline	2.72 ±0.28	2.58 ±0.38	0.360
	15 days	2.32 ±0.33	1.96 ±0.14	0.006*
	p-value	0.001*	0.001*	–
CRP level [mg/L]	baseline	2.50 ±0.40	2.38 ±0.36	0.300
	15 days	2.40 ±0.40	2.02 ±0.32	0.006*
	p-value	0.004*	0.002*	–

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

Table 2 shows the mean counts of CFUs at baseline and at 15 days of intervention. The Wilcoxon signed-rank test results showed statistically significant differences in the mean counts of CFUs in the post-intervention period as compared to baseline in both groups ($p = 0.001$). Hence, we can infer that both the nanocomposite mouthwash and the CHX mouthwash showed similar effects of significantly reducing the mean CFU counts. Table 3 outlines the results of the Mann–Whitney U test, which revealed no statistically significant difference between group A and group B at baseline. However, after 15 days of intervention, the difference between the 2 study groups in the mean count of CFUs showed statistical significance ($p = 0.001$).

Table 2. Comparisons of the mean counts of colony-forming units (CFUs) at different time intervals within each study group (Wilcoxon signed-rank test)

Study group	Time interval	*M* ±*SD*	min	max	Z	*p*-value
Group A	baseline	16,047.00 ±1865.39	15,756.00	20,123.00	–3.40	0.001*
	15 days	620.66 ±185.10	380.00	980.00		
Group B	baseline	15,840.66 ±1742.90	12,450.00	15,840.66	–3.50	0.001*
	15 days	324.66 ±108.81	180.00	540.00		

min – minimum; max – maximum; * statistically significant.

Table 3. Comparisons of the mean counts of colony-forming units (CFUs) at different time intervals between the 2 study group (Mann–Whitney *U* test)

Time interval	Study group	*M* ±*SD*	min	max	Z	*p*-value
Baseline	group A	16,047.00 ±1865.39	15,756.00	20,123.00	–4.66	0.060
	group B	15,840.66 ±1742.90	12,450.00	15,840.66		
15 days	group A	620.66 ±185.10	380.00	980.00	–3.86	0.001*
	group B	324.66 ±108.81	180.00	540.00		

* statistically significant.

Discussion

The present study compared the antibacterial and anti-inflammatory efficacy of the amla seed-mediated GO-Ag nanocomposite mouthwash and the CHX mouthwash in plaque-induced gingivitis patients. Nanoparticles kill bacteria by creating reactive oxygen species (ROS), such as hydrogen peroxide, superoxide radicals and singlet oxygen. Reactive oxygen species are the products of oxygen metabolism in physiological settings, and play an important role in cell signaling and cellular homeostasis. When metal oxide NPs are in an aqueous medium, they gradually release metal ions. These metal ions can permeate cell membranes, and interact with nucleic acids and protein functional groups. Such interactions have a variety of consequences, including abnormal enzyme activity, cell structure changes, the modification of physiological processes, and microorganism inhibition.[20] Chlorhexidine gluconate is considered a standard agent for chemical plaque control. However, its long-term use can have several undesirable side effects. Therefore, the authors of the present study explored the use of a nanocomposite mouthwash as an alternative to a CHX mouthwash.

This study is unique, since there are no previous studies on the antibacterial and anti-inflammatory efficacy of the amla seed-mediated GO-Ag nanocomposite mouthwash. In the present study, it was observed that both the nanocomposite and CHX mouthwashes showed similar effects in terms of reduction of the PI and GI scores. Pradeep et al. carried out a randomized controlled trial to evaluate the efficacy of the triphala (TRP) mouthwash in reducing plaque and gingivitis, and observed that the TRP and CHX mouthwashes were equally effective in improving PI, GI and the simplified oral hygiene index (OHI-S) at all time intervals,[21] which is in accordance with the current study. In another study, the turmeric and CHX mouthwashes were used as adjuvants to mechanical plaque control[22]; the findings were in agreement with the results of the present study. Similar results were reported by Ramamurthy et al.[23] Thus, our findings demonstrate that the amla seed-mediated GO-Ag nanocomposite mouthwash possesses anti-plaque and anti-gingivitis properties.

Antibacterial properties were also assessed by evaluating CFUs, with the results showing that the nanocomposite and CHX mouthwash groups had equal significant reductions in the mean CFU count. Komariah et al. assessed the effects of nano chitosan and nano calcium, derived from *Xylotrupes gideon*, in comparison with the CHX mouthwash.[24] Their results were in line with the present study, where both the test and control mouthrinses had comparable potential to reduce total bacterial colonies in the oral cavity. In another study, the authors compared the effects of the GO NP mouthwash and the GO-sodium fluoride (NaF) nanocomposite mouthwash on the *Streptococcus mutans* (*S. mutans*) count in the saliva of rats.[25] They reported that both mouthwashes efficiently reduced *S. mutans* in the saliva of rats as compared to the control group.

The mechanism of action behind the antibacterial property of GO involves physical damage to the cell membrane, oxidative stress, and entrapment or wrapping. Furthermore, GO compromises the cell membrane and cell wall integrity. The modified functional groups on GO NPs are thought to play a key role in modulat-

ing oxidative stress. Despite this, the precise correlation of functional groups with antibacterial activity has not been well elucidated due to the physical and chemical complexities of GO. Zhao et al. investigated the antibacterial activity of functionalized GO sheets (40 g/mL) on *S. mutans*.[26] The effects of the GO sheet on *S. mutans* biofilms and *S. mutans* in a planktonic form were dose-dependent, according to the researchers. Furthermore, GO functional groups were important for the antibacterial action.[26] Thus, our findings show that the amla seed-mediated GO-Ag nanocomposite mouthwash possesses an antibacterial property.

C-reactive protein is an acute-phase reactant produced in response to a variety of inflammatory stimuli, such as trauma, infection, heat, and hypoxia. It has a wide range of actions, including pro-inflammatory properties, complement factor activation, the neutralization of the invading pathogens, and tissue regeneration. Periodontitis patients have considerably greater levels of CRP in their serum and GCF than non-periodontitis subjects.[27] In addition, there is growing evidence that effective periodontal treatment can reduce the CRP levels. As GO NPs and AgNPs have been proven to have antioxidant and anti-inflammatory properties, they can regulate the CRP levels in GCF.[15] Since GCF reflects the ongoing changes in the periodontium, the evaluation of the CRP levels at gingival crevicular sites can be considered a more accurate and less invasive method. In the present study, there were statistically significant differences in the CRP levels in GCF at baseline and at the 15-day follow-up in both study groups. Kumar et al. evaluated the effect of non-surgical periodontal therapy on the CRP levels in GCF, and observed that it was effective in lowering the CRP levels,[28] which is in accordance with the present study. Furthermore, the findings of the study supported the hypothesis that the levels of GCF biomarkers are related to the degree of inflammation, collagen degradation and bone turnover.[28] In another study, both scaling and root planing (SRP) alone and using a diode laser as an adjunct showed a reduction in the serum CRP levels,[29] which is in agreement with the results of the present study. A study by Jayaprakash et al. reported that the CRP levels in GCF gradually increased from a healthy periodontium to gingivitis, and further increased in periodontitis.[30] Moreover, it was stated that periodontal therapy reduced the CRP levels.[30] Thus, our findings show that the amla seed-mediated GO-Ag nanocomposite mouthwash possesses an anti-inflammatory property.

One of the advantages of the present study is that the baseline parameters were standardized, reducing the impact of gingivitis severity on the effectiveness of the oral rinses. However, this study has some limitations, such as a small sample size and a short follow-up period (15 days). In order to substantiate the study results, a study with a larger sample size and a longer follow-up period should be conducted.

Conclusions

The amla seed-mediated GO-Ag nanocomposite mouthwash efficiently reduced plaque, gingival inflammation and CFUs among patients with plaque-induced gingivitis, but was not equivalent to the CHX mouthwash. Thus, the amla seed-mediated GO-Ag nanocomposite mouthwash can be considered an alternative to the CHX mouthwash.

Ethics approval and consent to participate

The study was approved by the institutional ethics committee at Saveetha Dental College and Hospitals, Chennai, India (IEHC/PERIO-18/2022/35). 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

Oral health and caries risk profile assessment using the the Cariogram in thalassemia patients with or without splenectomy: A cross-sectional study

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Abstract

Background. Splenectomy is performed in β-thalassemia cases due to the destruction of red blood cells (RBCs), and the consequent splenomegaly.

Objectives. The aim of the present study was to compare oral health and the caries risk between β-thalassemia patients with or without splenectomy, using the Cariogram.

Material and methods. In a cross-sectional study carried out in both the Maternity and Children Hospital and the King Fahad Hospital, Al-Madinah al-Munawwarah, Saudi Arabia, interviews, salivary sampling and oral clinical examinations were performed on 60 children and adolescents with β-thalassemia major (mean age: 13 ±3 years; 65% with splenectomy). The Cariogram program was used to calculate the caries risk. The main outcome measures were the number of decayed, missing due to caries, and filled teeth (DMFT), plaque and gingival indices, and the caries risk.

Results. Of individuals with and without splenectomy, tooth brushing was reported in 49% and 57%, respectively ($p > 0.05$). Individuals with splenectomy had lower plaque and gingival bleeding scores ($p \leq 0.05$). Salivary secretion was identical in both groups. Caries experience and the caries risk were higher in individuals without splenectomy ($p > 0.05$ and $p \leq 0.05$, respectively).

Conclusions. Within the study limitations, children and adolescents with β-thalassemia had high plaque and gingival bleeding scores, as well as caries experience and caries risk. Those with splenectomy demonstrated lower figures than those without. Individuals with β-thalassemia, particularly those with splenectomy, need to be educated about the oral side effects of the disease and its treatment.

Keywords: oral health, splenectomy, caries risk, beta thalassemia

Introduction

Beta-thalassemia is a common genetic disorder distinguished by the absent or decreased synthesis of globin protein chains, particularly the beta and alpha chains, resulting in a reduced production of hemoglobin and hypochromic microcytic anemia.[1] Spleen enlargement, abnormal red blood cells (RBCs), iron overload, and other changes occur because of the increased rate of hemopoiesis observed in a number of conditions, including β-thalassemia.[2,3]

It is well-established that individuals with β-thalassemia are at a higher risk of infection, particularly those who have undergone splenectomy.[1] Regarding oral health, several studies have observed a high dental caries index in β-thalassemia patients,[4] which may be attributed to several factors, including the hematologic condition itself, medications, the altered tooth morphology, and the modified salivary composition.[5] Other oral manifestations related to periodontal health, occlusion and the maxillary growth have also been reported.[6–8]

As dental caries is known to be multifactorial in nature, different factors, such as the systemic condition of the individual, may contribute to the disease.[9] Thus, the multifactorial management of dental caries has continuously been advocated and recommended in the literature.[10] Moreover, assessing the individual's caries risk plays a vital role in its prevention and management.[11–13] Several methods and tools have been used for multifactorial caries risk assessment, one of which is the Cariogram,[14] a computer program that calculates the relationship between dental caries and many possible protective and invading factors in an attempt to illustrate the caries risk and provide feasible explanations and recommendations for the individual patient.[14]

To date, no studies have investigated the association between splenectomy and the oral health and the caries risk in patients with β-thalassemia. Therefore, the present study aimed to evaluate the oral health and caries risk profiles in β-thalassemia patients, using the Cariogram, and to compare them in patients with or without splenectomy.

Material and methods

Study design and sample

This observational cross-sectional analytical investigation was approved and registered by the Taibah University College of Dentistry Research Ethics Committee (approval No. TUCDREC/20180107/Qarah) and the Institutional Review Board at the General Directorate of Health Affairs in Al-Madinah al-Munawwarah, Saudi Arabia (approval No. 120–07/02/2018). The study followed the Declaration of Helsinki guidelines with regard to involving human participants[15] and the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist for reporting observational studies.[16]

The study population comprised children with β-thalassemia major attending the hematology/oncology center at the Maternity and Children Hospital and adolescents with β-thalassemia major attending the specialized clinic at the King Fahad Hospital in Al-Madinah al-Munawwarah, Saudi Arabia.

Study outline

All participants underwent a personal interview, had their saliva sampled and were subjected to an oral clinical examination during routine blood transfusion appointments. Those who could not be examined during the same visit were given an appointment at the Taibah University Dental College and Hospital. The interview collected general background information, including age, gender, nationality, oral hygiene practices, the type of thalassemia, the medications used, a history of splenectomy, and other health-related aspects. Child participants had their guardians answer the interview questions.

Saliva sampling

The participants washed out their mouths with distilled water before starting the procedure. Unstimulated saliva was collected by a standard spit method, with the participant in an upright sitting position and inclined to the front, spitting for 5 min into a graded plastic container.[17] The samples were used to measure the salivary secretion rate of each participant. Sampling was not possible in 2 of the participants.

Oral clinical examination

Oral clinical examinations followed standard infection control precautions, were performed on a portable dental chair under an optimal light source, and utilized a graded periodontal probe (Hu Friedy, Chicago, USA), a mouth mirror and sterile cotton pads. The recorded parameters included the number of decayed, missing due to caries, and filled teeth (DMFT), the gingival index (GI),[18] and the plaque control record (PCR).[19] The DMFT was recorded for all teeth, and GI and PCR were registered at 4 tooth sites (mesial, distal, buccal, and lingual). Two crossed upper and lower quadrants were selected for GI and PCR by coin flip.

Caries risk assessment

The Cariogram software, v. 3.0 (Malmö University, Sweden) assessed the participants' caries risk. The risk is calculated by entering 9 related factors into the software, including caries experience, systemic diseases, the plaque

score, diet content, diet frequency, the fluoride program, salivary secretion, buffer capacity, and the salivary *Streptococcus mutans* counts.[14] To compensate for a lack of laboratory analysis, a reduced Cariogram model was adopted, where variables that relied on the salivary analysis were excluded.[20] Upon variable entry, the Cariogram produces a graphical pie chart that shows how the entered variables interact. The green sector of the pie chart, named "Actual Chance (%) to Avoid New Cavities" (Chance-AC), reflects the individual's caries risk. The smaller the green sector, the higher the risk of developing new caries lesions in the future.[14]

Statistical analysis

Descriptive statistics in the form of mean and standard deviation (*M* ±*SD*) were used to describe continuous variables, and frequencies represented categorical variables. Given the unequal data distribution, the Mann–Whitney *U* test compared differences in continuous outcome variables between individuals with or without splenectomy, while the χ^2 test was used for categorical variables. As most of the study participants fell within the 'very high' caries risk category according to the Cariogram, i.e., Chance-AC = 0–20%,[21] a decision was made to highlight those with Chance-AC <10% as the 'highest' caries risk among individuals with or without splenectomy. A *p*-value ≤0.05 was considered statistically significant. Data was analyzed using IBM SPSS Statistics for Windows, v. 20.0 (IBM Corp., Armonk, USA).

Ethical considerations

Written informed consent was obtained from the participants (or their parents) before their inclusion in the study. The purpose of the study was explained to each participant, and they were assured that participation was voluntary, with no negative repercussions in terms of the quality of health care offered due to not participating. The participants were also assured that all information would be kept confidential and would only be used for scientific purposes. They were informed of any need for treatment based on the study findings.

Results

A total of 60 individuals with β-thalassemia major were involved in this study, including all children followed up at the Maternity and Children Hospital (*n* = 40; 67%) and adolescents attending the clinic at the King Fahad Hospital (*n* = 20; 33%). The mean age was 13 ±3 years; 53% of the participants were males and 47% were females. Thirty-nine (65%) participants had undergone splenectomy (Table 1).

Of the individuals who had undergone splenectomy, only 49% reported brushing their teeth, while 57% of those without splenectomy brushed their teeth ($p > 0.05$) (Fig. 1). High dental plaque and gingival bleeding scores were recorded in both groups, with individuals without splenectomy demonstrating significantly higher scores ($p \leq 0.05$) (Table 1).

Salivary secretion was within the normal range, and identical in both groups ($p > 0.05$) (Table 1). Individuals without splenectomy a higher DMFT score than those who had undergone splenectomy (5.5 ±5 vs. 4.5 ±5, $p > 0.05$) (Table 1).

Both individuals with and without splenectomy demonstrated 'very high' caries risk profiles, as illustrated by the Cariogram, with a low Chance-AC of 17 ±12% and

Table 1. Demographics, salivary secretion, oral health clinical parameters, and caries risk in β-thalassemia patients with and without splenectomy

Variable	β-thalassemia patients			*p*-value
	with splenectomy (*n* = 39)	without splenectomy (*n* = 21)	total (*N* = 60)	
Age [years] (*M* ±*SD*)	13 ±8	15 ±8	13 ±3	0.177
Gender (M/F) *n* (%)	18 (46)/21 (54)	14 (67)/7 (33)	32 (53)/28 (47)	0.129
Saliva secretion rate [mL/min] (*M* ±*SD*)	2.3 ±1.0	2.3 ±1.0	2.6 ±1.0	0.868
PCR [%] (*M* ±*SD*)	60 ±31	79 ±22	67 ±30	0.020*
GI [%] (*M* ±*SD*)	33 ±27	48 ±23	38 ±27	0.027*
DMFT (*M* ±*SD*)	4.5 ±5.0	5.5 ±5.0	4.9 ±5.0	0.434
Chance-AC [%] (*M* ±*SD*)	17 ±12	14 ±14	16 ±13	0.046*

M – mean; *SD* – standard deviation; M – male; F – female; PCR – plaque control record; GI – gingival index; DMFT – number of decayed, missing due to caries, and filled teeth; Chance-AC – "Actual Chance to Avoid New Cavities" according to the Cariogram.

14 ±14%, respectively ($p \leq 0.05$) (Table 1). Seventy-six percent of the total sample had Chance-AC ≤ 20%, while 45% had Chance-AC < 10% (data not shown). Figure 2 presents the distribution of individuals from both groups according to Chance-AC <10% and ≥10%, with more individuals from the group who had not undergone splenectomy falling into the higher risk category ($p \leq 0.05$).

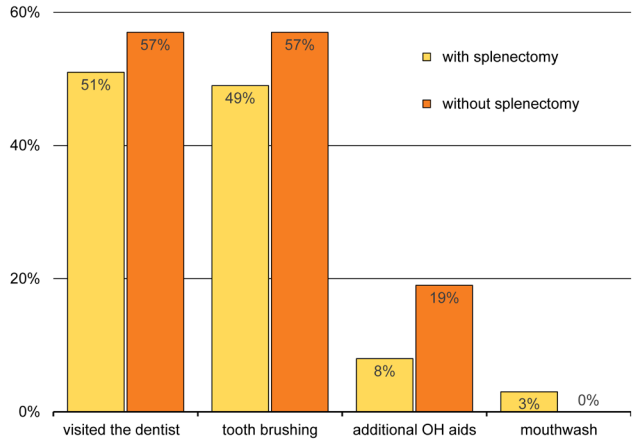

Fig. 1. Bar chart showing no significant differences between β-thalassemia patients with and without splenectomy in terms of oral hygiene habits and dental visits ($p > 0.05$; χ^2 test)

OH – oral hygiene.

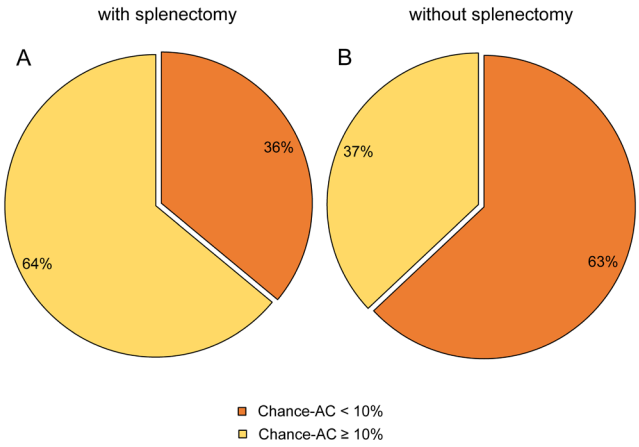

Fig. 2. Pie charts illustrating the "Actual Chance to Avoid New Cavities" (Chance-AC) according to the Cariogram in β-thalassemia patients

A – patients with splenectomy; B – patients without splenectomy. Significantly more individuals with Chance-AC < 10%, i.e., the 'highest' caries risk category, were observed in the non-splenectomy group ($p \leq 0.05$; χ^2 test)

Discussion

In this study, around half of the participants with β-thalassemia major visited the dentist and brushed their teeth, with no significant differences observed between those with or without splenectomy. These alarming figures may be due to the fact that patients with β-thalassemia (and their families) are often occupied with dealing with the disease and its devastating repercussions, which may lead to oral health negligence.[22] Moreover, certain oral features in individuals with β-thalassemia, such as tooth malposition and malocclusion, may hinder the maintenance of good oral hygiene.[7,23]

The present study demonstrated that β-thalassemia patients had high plaque and gingival bleeding indices, which is in line with the literature on oral findings in people with β-thalassemia major, and is likely related to local factors, such as malocclusion, protruded front teeth, and consequently, an anterior open bite and xerostomia.[24]

The salivary flow rate was generally within the normal range, with no significant differences between individuals with or without splenectomy, which was contrary to expectations, since individuals with β-thalassemia usually demonstrate iron deposits due to iron overload,[25] related to a decreased salivary flow rate, pain and swelling.[26] However, a histological study involving 15 adults with thalassemia failed to demonstrate a clear correlation between salivary secretion impairment and the destruction of the salivary glands by iron deposition, lymphocyte infiltration and fibrosis, warranting further research in this area.[27]

The current study is the first to assess the caries risk in individuals with β-thalassemia. As dental caries is a multifactorial disease, the Cariogram computer software was used to estimate the risk by illustrating how different caries-related factors interact.[14] Individuals with β-thalassemia major demonstrated high caries risk, which may be attributed, in part, to the high caries experience observed. Other contributory factors include improper dietary habits, bone deformities, and lack of appropriate oral health-related knowledge.[23] In addition, social status reflects significantly on oral health, and many of these families could not afford the high costs of dental treatment.[28]

Patients with β-thalassemia major who had undergone splenectomy had significantly lower plaque and gingival bleeding index scores, a lower DMFT score, and lower caries risk than those without splenectomy. Beta-thalassemia patients who undergo therapeutic splenectomy due to massive splenomegaly are more susceptible to bacteremia and the post-splenectomy sepsis caused by gram-negative bacteria.[23] With an increased chance of infection, β-thalassemia patients who undergo splenectomy receive antibiotic therapy for life, usually in the form of oral penicillin, with some also receiving a combination of different prophylactic injections pre-operatively.[29,30] This may explain the differences in oral health findings between β-thalassemia patients with and without splenectomy. The effects of penicillin and antibiotics on dental caries in general have been reported since the 1960s.[31] Similarly, periodontal disease, induced mostly by microbial plaque, has been treated by antibiotics alone or as adjuncts to mechanical plaque removal.[32] However, antibiotic administration for oral diseases remains debatable due to obvious side effects and the risk of antibiotic resistance, and is limited to very specific cases.

Limitations

The small sample size may raise questions regarding the representativeness of the study findings. However, this could not be avoided, as individuals with β-thalassemia regularly visiting the clinic for follow-up represent a limited population. The intention was to include all possible patients from the database, but this was not achieved due to unprecedented circumstances. Nevertheless, findings from the current investigation may add significant value to the literature related to such a rare disease.

A reduced Cariogram model was used to assess the caries risk of the studied sample. It can be argued that each factor entered into the Cariogram software is significant for accurately determining the individual's risk. However, using a reduced Cariogram has been reported in the literature[33] and can be of practical use in cases with limited laboratory resources.

Conclusions

Within the study limitations, it can be concluded that individuals with β-thalassemia major had high plaque and gingival bleeding scores, caries experience, and risk of future caries, as shown by the Cariogram. Individuals who had undergone splenectomy presented significantly lower values than those without splenectomy.

Practical significance

All individuals with β-thalassemia, with or without splenectomy, should have regular dental visits to carry out appropriate preventive and therapeutic measures that address the causative and contributory factors of oral disease. Affected children and their parents need to be supported, educated, and made aware of the side effects of the disease and its treatment, including subclinical or masked oral health conditions.

Ethics approval and consent to participate

The study was approved and registered by the Taibah University College of Dentistry Research Ethics Committee (approval No. TUCDREC/20180107/Qarah) and the Institutional Review Board at the General Directorate of Health Affairs in Al-Madinah al-Munawwarah, Saudi Arabia (approval No. 120–07/02/2018). All participants or their parents 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

Assessment of knowledge, practices and attitudes of dentists toward coronavirus disease while performing aerosol-generating procedures in dentistry: A cross-sectional survey from India

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Abstract

Background. Dental procedures that generate aerosols, if not performed with precautions, are the source of infection during the coronavirus disease 2019 (COVID-19) pandemic.

Objectives. The aim of the present study was to analyze dentists' awareness, knowledge and practice regarding the spread of coronavirus in India, to fill the knowledge gaps and to limit the spread of the virus while performing aerosol-generating procedures (AGPs).

Material and methods. A cross-sectional survey containing 23 close-ended questions was conducted using the Google Forms platform. The questionnaire was knowledge, practice and attitude-based (KPA). It was completed by Indian dentists stratified according to gender, designation, years of experience, and residence type. Data was analyzed with the χ^2 test, and summarized using frequency and percentage. The independent samples t test and the one-way analysis of variance (ANOVA) were used to analyze intergroup differences.

Results. With regard to knowledge, females, specialists with 10 years of experience and those living in metro cities scored higher. For practice-based questions, males, the teaching staff, dentists with less than 5 years of experience, and those living in suburban areas provided more correct answers. For attitude-based questions, females, general practitioners (GPs), dentists with less than 5 years of experience, and those residing in suburban areas provided more correct answers.

Conclusions. Dentists in India demonstrated a high level of understanding with regard to COVID-19. However, they showed a limited understanding of the extra preventative procedures to safeguard against the disease. The study findings have significant implications for the measures aimed at increasing dentists' level of KPA toward coronavirus.

Keywords: knowledge, practice, attitude, coronavirus, aerosol-generating procedure

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Introduction

The fast-spreading coronavirus has been affecting global society, economics, health, and lifestyle since December 2019.[1] Since the first reported case in Wuhan, China, coronavirus has gradually encompassed the entire globe via close human-to-human contact.[2,3] Irrespective of age, gender or nationality, the deadly virus can infect everyone, causing multiple respiratory disorders, with symptoms ranging from a mild cough to life-threatening pneumonia, acute respiratory distress syndrome (ARDS), and multi-organ dysfunction leading to organ failure and subsequent death.[2] Inhalation and contact with contaminated droplets are the 2 ways of disease transmission, and the incubation period ranges from 2 to 14 days.[3] However, symptoms usually occur after 2–4 days of the incubation period. They include fever, cough, a sore throat, dyspnea, exhaustion, and malaise.[2] In most people, the infection is minor or asymptomatic, but in the elderly and those with comorbidities, it may be lethal.[2] The relative death rate is expected to be 2–3%.[2]

The virus can be detected in respiratory secretions with the use of specific molecular assays. An increased white blood cell (WBC) count and a high C-reactive protein (CRP) level are the initial laboratory test results that can help detect the disease.[4] Until July 2022, 546 million confirmed cases and 6.3 million deaths were recorded worldwide.[5] India reported 43.4 million confirmed cases and 0.5 million deaths.[6] Maharashtra, Kerala, Karnataka, Tamil Nadu, and Andhra Pradesh are the top 5 states affected, accounting for 56% of all cases.[7] Throughout this worldwide health disaster, dental professionals are at high risk of infection, as they are in direct contact with their patients.[3] In addition, devices such as ultrasonic scalers, air-water syringes and air turbine handpieces, commonly used in routine dental treatment, can be contaminated with the patient's saliva and blood, resulting in the production of infectious aerosols.[8]

Throughout the coronavirus disease 2019 (COVID-19) pandemic, the participation of dental professionals was critical in raising coronavirus awareness among themselves and the community.[9,10] As such, dental workers must have a high degree of coronavirus understanding, and a proper attitude toward infection control measures and disease significance. With this in mind, a survey was created to evaluate dentists' knowledge, practices and attitudes regarding coronavirus and its impact on dentistry.[11] The purpose of the present study was to analyze dentists' awareness and knowledge of coronavirus and infection control strategies in the dental context in India, to fill the knowledge gaps, and to limit the spread of the virus while performing aerosol-generating procedures (AGPs) in dentistry.

Material and methods

The present online cross-sectional survey was developed in February 2022. It included 23 questions regarding AGPs in dentistry (Table 1). The respondents were encouraged to fill in the questionnaire over the Google Forms platform. It made the data collection process simpler, quicker and safer with regard to the COVID-19 pandemic, as the Indian government had advised the public to avoid face-to-face interaction and isolate at home. Individuals were sent a link to Google Forms that explained the study background and purpose, the voluntary nature of participation, and contained the declarations of confidentiality and privacy, and instructions for completing the questionnaire. The study was open to all Indian dentists working in private clinics, teaching faculties, medical facilities, or hospitals. The Dental Council of India (DCI) website lists all dentists currently registered in India. From the website, we randomly searched 3,500 dentists who were available on social media, such as Facebook, Instagram, Twitter, and WhatsApp, and requested them to participate in the survey. However, only 2,367 dentists agreed to take part. The 2,367 registered dentists who expressed interest in the survey were provided with an online link via the same relevant social media platform. Of the 2,367 respondents, only 1,279 met the eligibility criteria (Table 2). To create a balance of gender-based replies and prevent any bias in the demographic data, another 279 respondents from male dentists were randomly excluded.

The questionnaire was developed after reviewing the published literature and the most recent COVID-19 information available from the Centers for Disease Control and Prevention (CDC) dental setting guidelines,[11–15] and was also assessed for validity by 5 experienced reviewers. The survey consisted of 23 questions regarding AGPs (safety majors, the hygiene protocol and risk perceptions) in the clinic and broadly divided into 3 categories: attitude-based (question numbers 1–6); practice-based (question numbers 7–15); and knowledge-based (question numbers 16–23). The survey was designed as a multiple-choice questionnaire with 1 correct option for each question. The number of responses to all options was recorded for each question. The answers were analyzed according to gender (male or female), designation (a general practitioner (GPs) – BDS (Bachelor of Dental Surgery), a specialist – MDS (Master of Dental Surgery) or the teaching staff, i.e., BDS/MDS), years of experience (5, 5–10 or >10) and residence type (a metro city, a suburban area or a rural area). Ethical approval for the study was granted by the ethics committee at the Teerthanker Mahaveer Dental College and Research Centre, Moradabad, India (No. TMDCRC/IEC/SS/21-22/PRO 01).

Table 1. Participants' responses to attitude-, practice- and knowledge-based questions

Section	Questions	Answers	Percentage [%]
Attitudes	1. Do you feel safe while performing AGPs?	maybe	28.4
		no	33.9
		yes	37.7
	2. Do you strictly follow the WHO hand hygiene protocol before treating a new patient?	maybe	6.2
		no	3.6
		yes	90.2
	3. Which of the following is important before treating a patient?	negative RT-PCR	5.0
		proper history taking	10.3
		none	0.8
		both	**83.9**
	4. On a scale from 1 to 10, with 10 being the highest score, rate the following: Do you consider the usage of a PPE kit in an asymptomatic patient while performing AGPs to be important?	**10**	**88.0**
	5. What measures have you taken to minimize the amount of produced aerosol?	replacing an air rotor with a micro-motor	14.6
		switching to non-contact laser procedures	6.3
		none	25.0
		both	**54.1**
	6. Do you use AC while performing AGPs?	always	8.2
		sometimes	46.3
		never	**45.5**
Practices	7. In which of the following clinical procedures, do you take maximum precautions?	AGPs	23.2
		history taking and clinical examination	9.0
		taking radiographs	1.3
		all	**66.5**
	8. What type of mask do you use while performing AGPs?	any	7.6
		double masking with a surgical mask	27.7
		a hand-sewn cloth mask	1.1
		a NIOSH-approved N95 mask	**63.6**
	9. Does your clinic have a separate triage area for onitoring vitals, like temperature, oxygen saturation and sanitization?	no	19.1
		planning to add in the future	21.0
		yes	**59.9**
	10. What extra safety measures do you take while screening patients?	checking their status with the Aarogya Setu application	3.8
		history taking over the phone before scheduling an appointment	23.0
		none	13.6
		both	**59.6**
	11. Which method do you use to sanitize metal objects in the clinic, like dental chairs, instruments, and other?	70% alcohol	58.3
		detergent wash	3.9
		diluted (5%) bleaching solution	**9.6**
		fumigation	23.8
		none	4.4
	12. What do you wear for personal protection while performing AGPs?	**a disposable PPE kit with a face shield, a mask and gloves**	**63.3**
		a surgical gown with a face shield, a mask and gloves	21.0
		a face shield, a mask and gloves	12.1
		a mask and gloves	2.9
		none	0.7
	13. Do you have a separate setup for donning and doffing the PPE kit?	no	30.7
		yes	**69.3**
	14. Do you change your PPE kit for consecutive patients when performing AGPs?	no	25.5
		yes	**74.5**
	15. Do you provide PPE kits to your patients and your assistant?	maybe in the future	16.7
		no	14.9
		only to the assistant	28.6
		only to patients	3.8
		yes	**36.0**

Section	Questions	Answers	Percentage [%]
Knowledge	16. What is the correct sequence of donning the PPE kit?	**gloves, a mask, a gown**	**18.9**
		a gown, a mask, gloves	59.2
		a mask, gloves, a gown	3.6
		a mask, a gown, gloves	18.3
	17. What is the correct sequence of doffing the PPE kit?	gloves, a mask, a gown	31.3
		a gown, a mask, gloves	20.2
		a mask, a gown, gloves	9.8
		gloves, a gown, a mask	**38.7**
	18. Which of the following procedures is least safe to practice?	**crown preparation**	**37.8**
		impression making	26.4
		laser-guided procedures	26.9
		none	8.9
	19. What is the incubation period of SARS-CoV-2?	1–7 days	22.8
		1–15 days	**57.9**
		1–21 days	18.3
		1–35 days	1.0
	20. What is the duration of hand washing with soap/liquid soap?	10 s	3.9
		20 s	**25.8**
		30 s	42.8
		60 s	27.5
	21. The disease cannot be spread from asymptomatic patients	**false**	**83.0**
		true	17.0
	22. Which of the following investigations do you advise patients?	all	62.4
		blood investigations	0.6
		chest X-ray	1.2
		RT-PCR	**35.8**
	23. When should AGPs be appointed?	first appointment of the day	21.8
		anytime	30.1
		last appointment of the day	**48.1**

AGP – aerosol-generating procedure; WHO – World Health Organization; PPE – personal protective equipment; RT-PCR – reverse-transcription polymerase chain reaction; AC – air conditioning; NIOSH – National Institute for Occupational Safety and Health; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2. Correct answers are marked in bold.

Table 2. Inclusion and exclusion criteria

No.	Inclusion criteria	Exclusion criteria
1.	dentist practicing in India	dentist who declined to accept the consent form
2.	dentist who performs aerosol-generating procedures in their clinic	dental auxiliary
3.	dentist who has practised during the COVID-19 pandemic	dental practice that is limited to dental schools only
4.	–	incomplete or casually filled in forms

COVID-19 – coronavirus disease 2019.

Sample size calculation

The sample size was calculated using open-source software (OpenEpi) designed by CDC for epidemiological studies. Assuming a base population of 10,000 and setting the expected frequency of response at 59% (with a significance level of 0.1%), a sample size of 949 was required to achieve internal validity.

Statistical analysis

Descriptive and inferential statistics were analyzed by IBM SPSS Statistics for Windows, v. 20.0 (IBM Corp., Armonk, USA). The data was analyzed using the χ^2 test, and summarized using frequency and percentage. The mean and standard deviation ($M \pm SD$) values were used to summarize clinical parameters (the knowledge,

practice and attitude scores between genders, designation types, experience categories, and residence types) for the groups. The independent samples *t* tests compared differences in knowledge, practices and attitudes between the gender groups. The one-way analysis of variance (ANOVA) assessed intergroup differences in knowledge, practices and attitudes between designation types, experience categories and residence types. Frequency and percentage were used to summarize qualitative data (individual responses to questionnaire items). Throughout the study, $p < 0.05$ was considered a statistically significant difference.

Results

Comparison between genders

To ensure an equal number of replies from each gender and prevent any bias in the gender-based data, 279 male responses out of 1,279 responses were randomly excluded from the research. Females scored higher on knowledge-based (42.03 ±17.07) and attitude-based questions (71.41 ±22.63), whereas males scored higher on practice-based questions (51.10 ±19.92) (Table 3).

Table 3. Assessment of knowledge-, practice- and attitude-based responses between genders

Section	Gender	*M* ±*SD*	*SEM*	*p*-value
Knowledge	males	37.53 ±17.71	0.90	0.001*
	females	42.03 ±17.07	0.69	
Practices	males	51.10 ±19.92	1.01	0.026*
	females	48.05 ±21.84	0.88	
Attitudes	males	63.69 ±26.63	1.35	0.001*
	females	71.41 ±22.63	0.91	

M – mean; *SD* – standard deviation; *SEM* – standard error of the mean; * statistically significant.

Comparison according to designation

General practitioners performed better on attitude-based questions (71.71 ±23.43), specialist dentists performed better on knowledge-based questions (43.17 ±19.96) and the teaching staff performed better on practice-based questions (54.39 ±20.67) (Table 4).

Comparison according to experience

The highest knowledge scores (51.14 ±13.75) were found among participants with more than 10 years of experience. The highest attitude scores (70.73 ±23.28) occurred among participants with less than 5 years of experience (Table 5).

Comparison according to the demographic area

Participants residing in metro cities had the highest knowledge scores (43.45 ±18.06), while suburban participants had the highest practice (52.25 ±20.80) and attitude scores (72.61 ±23.69) (Table 6).

Table 4. Assessment of knowledge-, practice- and attitude-based responses according to designation

Section	Designation	*M* ±*SD*	*SEM*	*p*-value
Knowledge	GPs	39.17 ±16.42	0.68	0.003*
	specialists	43.17 ±19.96	1.15	
	teaching staff	38.48 ±14.54	1.36	
Practices	GPs	49.23 ±21.85	0.90	0.009*
	specialists	47.26 ±19.64	1.13	
	teaching staff	54.39 ±20.67	1.93	
Attitudes	GPs	71.71 ±23.43	0.97	0.001*
	specialists	63.50 ±24.98	1.44	
	teaching staff	64.56 ±26.28	2.45	

GP – general practitioner; * statistically significant.

Table 5. Assessment of knowledge-, practice- and attitude-based responses according to experience

Section	Experience [years]	*M* ±*SD*	*SEM*	*p*-value
Knowledge	<5	39.59 ±17.18	0.57	0.001*
	5–10	44.42 ±20.49	2.54	
	>10	51.14 ±13.75	2.39	
Practices	<5	50.09 ±21.07	0.70	0.001*
	5–10	42.73 ±19.95	2.47	
	>10	38.38 ±21.17	3.68	
Attitudes	<5	70.73 ±23.28	0.77	0.001*
	5–10	51.54 ±28.25	3.50	
	>10	38.64 ±16.64	2.89	

* statistically significant.

Table 6. Assessment of knowledge-, practice- and attitude-based responses according to the demographic area

Section	Demographic area	*M* ±*SD*	*SEM*	*p*-value
Knowledge	metro city	43.45 ±18.06	1.15	0.001*
	suburban	39.67 ±17.15	0.68	
	rural	37.09 ±16.93	1.53	
Practices	metro city	44.49 ±20.21	1.28	0.001*
	suburban	52.25 ±20.80	0.83	
	rural	43.36 ±22.01	1.98	
Attitudes	metro city	60.28 ±23.70	1.50	0.001*
	suburban	72.61 ±23.69	0.94	
	rural	63.41 ±25.46	2.29	

* statistically significant.

Attitude-based questions

When performing AGPs, 66.1% of the participants felt safe, to some degree. The WHO hand hygiene protocol was followed by 90.2%, and 83.9% recognized that proper history taking and a negative reverse-transcription polymerase chain reaction (RT-PCR) test was vital before treating patients. Only 54.1% took all measures necessary to minimize the amount of produced aerosol, and air conditioning (AC) was never used by 45.5%; only 8.2% used it all the time (Table 1).

Practice-based questions

During clinical procedures, 66.5% preferred taking maximum precautions. The National Institute for Occupational Safety and Health-(NIOSH) approved N95 mask was used by 63.6%, and a separate triage setup was used by 59.9%. While screening patients, 59.6% took all extra safety measures. The most common method used for sanitizing metal objects was 70% alcohol, used by 58.3%. A disposable personal protective equipment (PPE) kit was used by 63.3%, and 69.3% had a separate setup for donning and doffing such kits, while a similar proportion changed the kit between patients. A total of 36.0% of dentists provided their patients and assistants with PPE kits (Table 1).

Knowledge-based questions

The correct sequence of donning the PPE kit was followed by 18.9%, whereas 38.7% chose the correct doffing sequence. Crown preparation was regarded as the least safe practice by 38.7%. The majority of the participants (57.9%) knew that the incubation period of the virus was 1–15 days. Regarding the hand washing procedures, 42.8% erroneously believed that 30 s with soap/liquid soap was safer than doing it for 20 s. Most participants (83.0%) knew that the virus can be spread from asymptomatic patients. Only 35.8% of the participants believed RT-PCR was a diagnostic coronavirus test. Meanwhile, less than half of the participants (48.1%) were aware that AGPs should be delayed until the last appointment of the day (Table 1).

Discussion

Coronavirus disease 2019 is currently a global issue, notably among healthcare professionals and patients. Dentists are particularly vulnerable to nosocomial infections and may be coronavirus carriers.[12,13] The goal of this study was to investigate the knowledge, practices and attitudes of dentists in India regarding coronavirus. A questionnaire was developed to fill the knowledge gaps and reduce the viral spread while performing AGPs in dentistry through proper precautions.

Dentists' knowledge level was generally excellent. However, the findings indicate that the participants had considerable gaps in their coronavirus understanding, since only 57.9% knew the approximate incubation period of the infection. The incubation period is believed to be up to 15 days; however, it can be longer in rare situations.[14–16] For dental professionals, this asymptomatic phase might be problematic as the disease can spread before any symptoms appear.[17]

Female dentists showed better knowledge and attitudes toward coronavirus than male dentists. Similar research was conducted by Sezgin and Çapan, who also found that females had better coronavirus knowledge.[18] However, Olum et al. reported contrary findings, showing that males had significantly more knowledge than females.[19] The different designations of dentists play a major role in determining how the overall practice was performed during the COVID-19 pandemic. While the teaching staff provided better practice-based responses and GPs provided more accurate answers to attitude-based questions, the specialized dentists gave more accurate knowledge-based responses.

Dentists with more than 10 years of experience had more knowledge than those with less than 5 years of experience, who in turn chose correct answers for practice and attitude-based questions. Our results are similar to those of Nour et al., who reported higher knowledge among healthcare workers who had practiced for more than 10 years.[20] However, their survey mostly included participants from Saudi Arabia and Pakistan, which differ from the Indian population.[20]

A thorough understanding of any disease helps in its early detection, diagnosis, treatment, and prevention, especially during a pandemic. There was a significant number of appropriate practices among the responders. This is in line with the observations made by Zhang et al.,[21] Saqlain et al.[22] and Huynh et al.,[23] who found ethical behavior among medical personnel throughout the COVID-19 epidemic. A high correlation between knowledge and practice suggests that all health professionals' understanding has to be improved to increase the adoption of preventative measures.[24]

In our study, dentists living in metro cities were more knowledgeable than suburban dentists. However, suburban dentists were better in practice management during the COVID-19 pandemic and had better attitudes toward safety. There is no previous demographic data available regarding practice.

For dental practitioners working in triage areas, all prevention measures are necessary, from social distancing and hand washing to protective equipment, such as face shields, masks, gowns, and gloves.[25,26] Manzar et al. concluded that all dental operations, whether they generate an aerosol or not, can disseminate the virus.[27] As a result, all safety precautions must be used equally. Most participants knew who to report

to in the event of unprotected contact with a known or suspected coronavirus-positive patient, and what to do if they developed symptoms. A similar result was observed in a study by Kamate et al., in which dentists were considered to have high grades in both knowledge and practice.[28] This demonstrates Indian dentists' understanding and their role in raising public awareness about infection control and prevention techniques. Furthermore, the study might generate curiosity among the participating dentists regarding the virus infection and its prevention, especially those who did not provide correct answers.

Despite the positive results of the study, there were a few limitations. Since it was a cross-sectional study, only associations, not cause-and-effect relationships, could be reported. In addition, it is important to consider the degree of ambiguity around the self-reporting component of the survey and the participants' memory.

Conclusions

In the case of the dental personnel, there is a substantial risk of spreading infectious illnesses. The occurrence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has presented dentists with new challenges and duties.[28,29] Dentists in India demonstrated a high level of understanding with regard to COVID-19. On the other hand, dentists showed a limited understanding of the extra preventative procedures to safeguard dental workers and patients against the virus. Our findings have significant implications for the measures aimed at increasing dentists' level of practice and strengthening preventative programs. Special precautions should be taken to prevent the illness spread among patients; they can also be used to manage other respiratory diseases in the future.

Ethics approval and consent to participate

Ethical approval for the study was granted by the ethics committee at the Teerthanker Mahaveer Dental College and Research Centre, Moradabad, India (No. TMDCRC/IEC/SS/21-22/PRO 01). 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

Management of recurrent aphthous ulcers with therapeutic Nd:YAG laser, using two different methods

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Abstract

Background. Low-level laser therapy (LLLT) has been applied for the management of craniomaxillofacial disorders, including intraoral wounds, as well as recurrent aphthous stomatitis (RAS) lesions. However, the proper combination of laser features and tissue characteristics remains the major challenge in the realm of photobiomodulation (PBM).

Objectives. The aim of the present study was to assess the feasibility of neodymium-doped yttrium aluminum garnet (Nd:YAG) laser therapy in treating RAS lesions, and to compare 2 techniques, different with regard to the distance between the fiber tip and the ulcer.

Material and methods. A total of 138 patients (94 males and 44 females) with untreated RAS were divided into 3 groups: focused laser (energy density: 48 J/cm^2; power density: 0.797 W/cm^2; spot size: 0.1256 cm^2); defocused laser (energy density: 21 J/cm^2; power density: 0.354 W/cm^2; spot size: 0.2826 cm^2); and placebo. In the focused group, laser irradiation was performed with the laser tip kept 1 mm away from the lesion. Acrylic cylinders were prepared to precisely fit the handpiece tip and hold it in the proper position. In the defocused group, acrylic cylinders were prepared to set the laser tip 6 mm away from the lesion to obtain defocused irradiation. Finally, in the placebo group, a routine laser therapy procedure was carried out with a helium-neon (He-Ne) red light laser. The lesion size, and pain intensity and duration were recorded.

Results. Photobiomodulation showed a significantly more efficient pain relief as compared to the placebo group ($p < 0.001$) and also significantly better results in decreasing pain duration ($p < 0.001$). Besides, the diameter of the lesions in the exposed cases decreased during the 3 consecutive days of the study, while an increase in the diameter of the lesions was noticed in the placebo group.

Conclusions. The Nd:YAG laser therapy, with the conditions and adjustments of the present study, may be successfully applied to manage RAS lesions, using either focused and defocused scanning techniques.

Keywords: lasers, aphthous stomatitis, solid-state, low-level laser therapies, oral medicine

Introduction

Recurrent aphthous stomatitis (RAS) is the most common ulcerative oral disorder, with recurring ulcers limited to the oral mucosa as the sole sign of the disease. Recurrent aphthous stomatitis is divided into 3 categories based on its clinical characteristics: minor ulcers; major ulcers; and herpetiform ulcers. Minor ulcers account for 80% of RAS cases, are less than 1 cm in diameter and tend to heal without leaving scars. Major ulcers have a diameter over 1 cm and delayed healing with scars. Herpetiform ulcers are regarded as a distinct clinical entity that presents as recurrent multiple small ulcers throughout the oral mucosa. The disease often appears for the first time during the 2nd decade of life. Pain and discomfort occur for at least 5 days in minor lesions to a maximum of 42 days in major aphthous ulcers.[1]

Recurrent aphthous stomatitis is currently treated in an empirical and non-defined manner. Local and symptomatic treatment modalities are the accepted procedures in simple cases of RAS. In more severe cases, topical therapies are still efficacious in accelerating the healing process, but fail to affect the attack intervals. Systemic immunomodulating factors, namely colchicine, pentoxifylline, prednisolone, dapsone, levamisole, thalidomide, azathioprine, methotrexate, cyclosporine A, interferon alpha, and tumor necrosis factor (TNF) antagonists, have been proven beneficial in resistant cases of major RAS, and have been indicated in aphthosis with systemic manifestations.[2] All therapies are palliative, and none results in permanent remission. Also, many medicaments, including thalidomide, dapsone, and colchicine, have shown various adverse reactions. In addition, corticosteroids have augmented the list of the local and systemic iatrogenic diseases with which both the clinician and the patient must deal.[3–5]

On the other hand, the metabolic, immunologic, analgesic, regenerative, and stimulative effects of photobiomodulation (PBM) are well known to the healthcare profession.[6–8] Much time has passed since it was discovered that therapeutic laser irradiation accelerated healing and decreased inflammation. Photobiomodulation has been used to manage craniomaxillofacial disorders, including intraoral wounds and RAS lesions. Helium-neon (He-Ne) (in the form of both gas and diode), therapeutic (defocused) carbon dioxide (CO_2), gallium arsenide (GaAs), erbium family, and therapeutic (defocused) neodymium-doped yttrium aluminum garnet (Nd:YAG) lasers have been used to treat RAS ulcers.[9–15]

The exact mechanism behind the impact of PBM is still unknown. However, several theories have been proposed. The adenosine triphosphate (ATP) hypothesis states that the cytochromes of mitochondria absorb light and convert it to energy.[16] Next, the cell is bioactivated and uses the energy to activate its intracellular structures. Meanwhile, the hyperpolarization following laser therapy confines neurotransmission and decreases the pain level.[17]

As Tunér and Hode previously stated in the preface of their book, things that require further clarification are the ideal doses, intensities, intervals of treatment, and other details in this delicate procedure.[18] The proper combination of laser features and tissue characteristics remains the major challenge in PBM. Thus, the current study aimed to assessed the feasibility of the therapeutic Nd:YAG laser treatment of RAS lesions, and to compare defocused and focused scanning.

Material and methods

A total of 243 patients were examined at the Dental School of Tehran University of Medical Sciences, Iran, over 2 years, and 138 patients (94 males and 44 females) met the inclusion criteria of this randomized clinical trial (ethical code: 3316). Sampling was accomplished using permuted blocks. The patients completed a questionnaire about their health condition and medical history. Only those without a systemic condition were considered for the study. Patients with untreated RAS developed within 48 h before the referral were also considered. In contrast, patients who had started taking medication, including mouthwashes, and patients with ulcerative colitis, Reiter's syndrome or Behçet's syndrome, herpetiform aphthous ulcers, and resistant RAS (non-responsive to treatment within the 3 weeks of the study), were excluded. Written informed consent was obtained from all patients after providing them with thorough instructions about the treatment procedure. Demographic data and examination outcomes were recorded for every patient. Study cases were then divided into 3 treatment groups: focused laser (F; n = 39), defocused laser (D; n = 46) and placebo (P; n = 28). Photobiomodulation was performed based on the parameters mentioned in Table 1.

Table 1. Photobiomodulation (PBM) parameters in the study groups

Group	Energy density [J/cm²]	Power density [W/cm²]	Spot size [cm²]
F	48	0.797	0.1256
D	21	0.354	0.2826

Groups: F – focused laser; D – defocused laser.

F: Focused laser group

The optical fiber was used focally, meaning the tip approached the lesion until a well-localized spot was obtained without touching the tissue. The laser tip was then moved helically toward the periphery of the lesion and irradiation was performed, leaving an unexposed safe margin of 1 mm around the lesion. To establish a uniform pattern of the helical movement, maintain the focal distance and avoid errors due to hand tremor, alginate impressions

were made from the laser handpiece, and an acrylic cylinder was fabricated for each patient. Acrylic cylinders were prepared to precisely fit the handpiece tip and hold it in the proper position (Fig. 1). The Nd:YAG laser (HighTech, Ravenna, Italy) was set as follows: fiber optic = 300 μ; TEM00; T = 60 s; F = 30 Hz; P = 3 W, and E = 100 mJ. If patients reported any sense of burning throughout the procedure, the movement speed was increased.

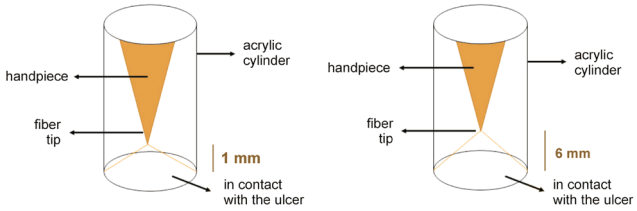

Fig. 1. Schematic presentation of 2 acrylic negatives of the laser tip – one with a 1-millimeter distance from the fiber tip to the base (tissue), and the other with a 6-millimeter from the fiber tip to the base (tissue)

D: Defocused laser group

The same protocol was followed, except that a defocused spot was maintained throughout the therapy. The helical movement was not necessary, though preferably used. Another acrylic cylinder was prepared to position the laser tip 6 mm away from the lesion to obtain defocused irradiation Fig. 1,2). The Nd:YAG laser was set as in group F.

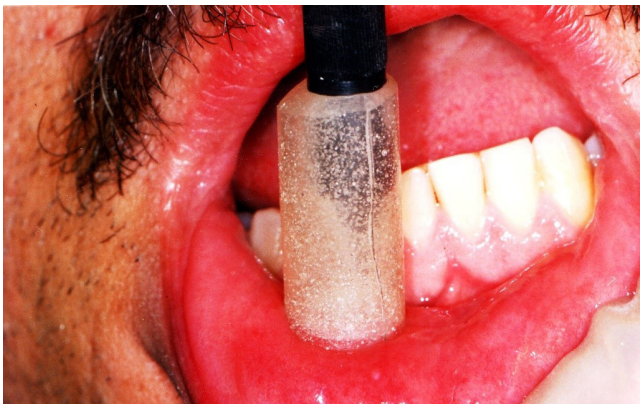

Fig. 2. Laser tip placed in the acrylic negative. The acryl base remains in contact with the aphthous ulcer and the 6-millimeter distance from the laser tip to the base renders the spot defocused. Laser irradiation is delivered through a helical movement

P: Placebo effect control

Patients were treated in the same way, but with a He-Ne red light laser (HighTech). The same movements were applied for the same duration as in the other 2 groups, but with a greater distance from the lesion (the cylinders were not used), and the laser beam was not aimed directly at the lesion.

For all groups, the PBM procedure was performed on the 1st, 2nd and 3rd day. The patients were asked for recall visits on the 7th, 14th, 30th, and 60th day of treatment (Fig. 3). Clinical presentations, including pain and the lesion size, were recorded at each visit. A periodontal probe was used to measure the lesion size (Fig. 4). Pain intensity and duration, the recurrence intervals of both pain and lesions, and the lesion diameter were also recorded at each visit based on the consented study scales scoring from 0 to 2.

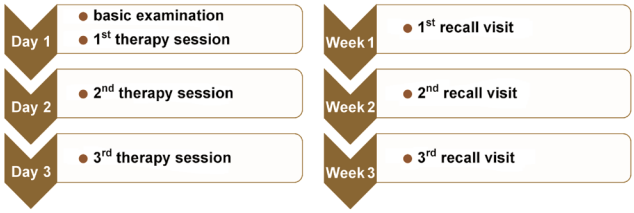

Fig. 3. Recall schedule before the monthly visits

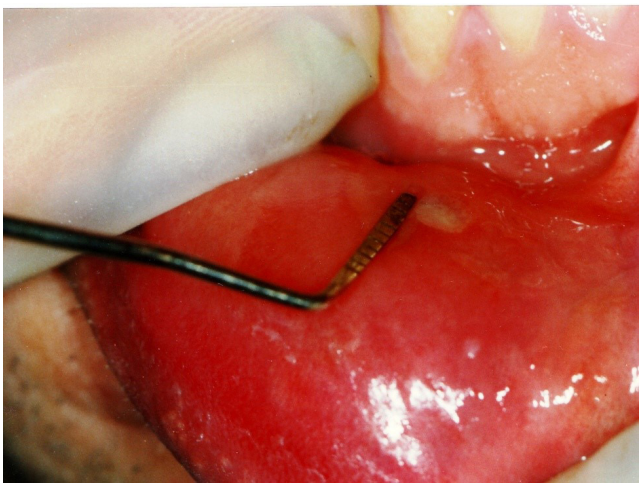

Fig. 4. Periodontal probe used for the measurement of the lesion diameter

Statistical analysis

The data was classified and statistically analyzed using SPSS for Windows, v. 14.0 (SPSS Inc., Chicago, USA), the χ^2 test and the one-way analysis of variance (ANOVA) ($p < 0.05$).

Results

The lesions typically developed in the 3rd, 4th and 5th decade of life (Fig. 5). The buccal vestibule was involved in 28.3% of cases, and the lip mucosa in 17.4%, whereas the tongue dorsum was the least involved (2.2%) (Fig. 6). Photobiomodulation proved significantly more efficient in terms of pain relief ($p < 0.001$) (Fig. 7), pain recurrence ($p < 0.001$) (Fig. 8), healing time ($p < 0.001$) (Table 2), and pain duration ($p < 0.001$) (Table 3) in comparison with the placebo. Also, a significant difference was found between the two PBM techniques when using Tukey's test ($p < 0.05$; 95% reliability),

with the defocused technique being more efficient in pain relief and decreasing lesion recurrence, and the focused technique resulting in a shorter healing time. The diameter of the lesions in the exposed cases decreased during the 3 consecutive days of the study. Concurrently, an increase in the lesion diameter was noticed in the placebo group. However, the differences were not statistically significant. Total and partial pain relief was noted in 53.2% and 23.4% of males, respectively. Meanwhile, 23.4% did not report any pain relief. The results in females were 50.0%, 18.2% and 31.8%, respectively. Gender ($p = 0.540$), age ($p = 0.430$) and the lesion site ($p = 0.090$) had no relationship with pain relief. Also, the lesion site was not associated with the healing time ($p > 0.05$); however, for pain duration, the tip of the tongue showed significantly more resistance than other sites, while the lip mucosa showed the least pain resistance (95% reliability).

None of the patients required supplemental topical anesthesia along with the laser irradiation. Some of the patients in group F reported a negligible stingy feeling. No adverse reactions due to the irradiation were noted.

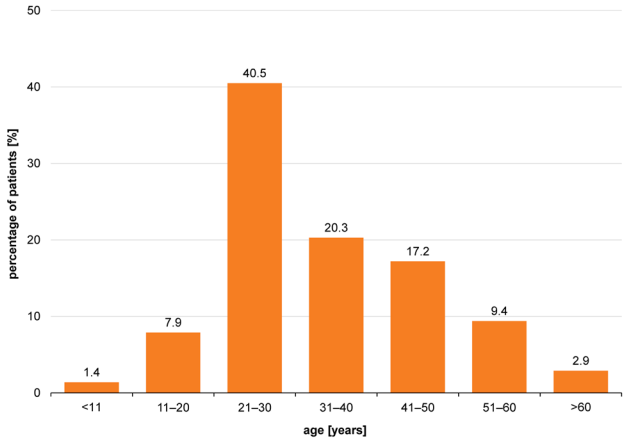

Fig. 5. Distribution of the patients based on age

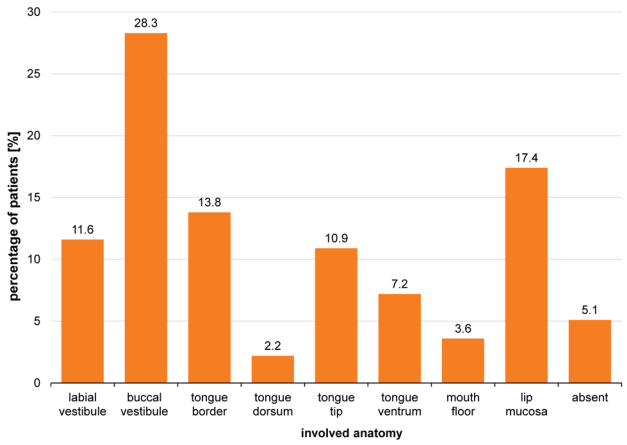

Fig. 6. Distribution of the patients based on the involved anatomy

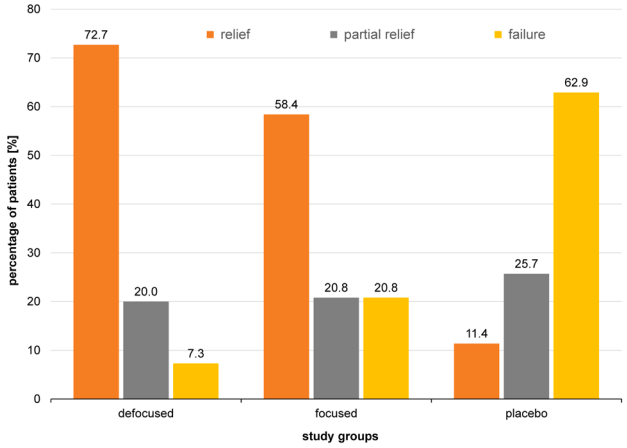

Fig. 7. Efficacy of laser therapy in terms of pain alleviation within the 3 study groups

A significant difference was found between the laser and placebo groups ($\chi^2 = 42.4$; $p < 0.001$). A significant difference was found between the 2 laser therapy techniques ($p < 0.05$; Tukey's test).

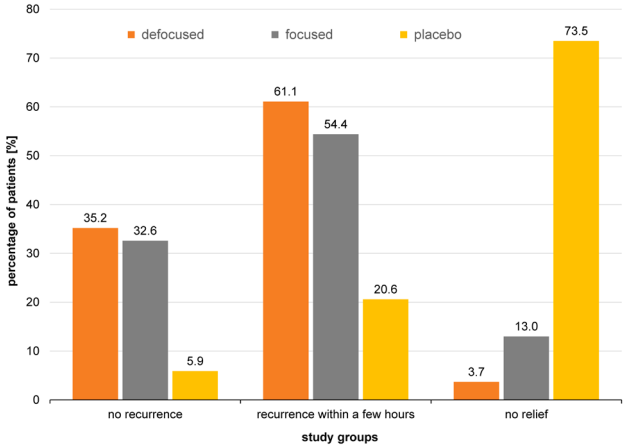

Fig. 8. Efficacy laser therapy in terms of pain recurrence within the 3 study groups

A significant difference was found between the laser and placebo groups ($\chi^2 = 60.2$; $p < 0.001$). A significant difference was found between the 2 laser therapy techniques ($p < 0.05$; Tukey's test).

Table 2. Healing time in the 3 study groups

Approach	*n*	Healing time [days]
Defocused	46	10.46 ±2.82
Focused	39	9.87 ±2.45
Placebo	28	12.54 ±3.40

Data presented as mean ± standard deviation ($M \pm SD$). A significant difference was found between the laser and placebo groups ($p < 0.001$). A significant difference was found between the 2 laser therapy techniques ($p < 0.05$; Tukey's test).

Table 3. Pain duration in the 3 study groups

Approach	*n*	Pain duration [days]
Defocused	46	6.15 ±1.97
Focused	39	6.89 ±2.50
Placebo	28	9.71 ±3.32

Data presented as $M \pm SD$. A significant difference was found between the laser and placebo groups ($p < 0.001$).

Most patients did not come back after the recall visits, so it was not possible to statistically analyze the recurrence. There were cases with a history of oral aphthous lesions recurring every 2 months among the study population, of which 1 returned after the recall and did not report any recurrences during 13 months. More noticeably, an 8-year-old boy with RAS recurring monthly (as reported by the parents) did not develop any lesions within 8 months of the therapy (group D) and was referred to our clinic to undergo further PBM (group D), with no lesions developing for the next 7 months. On the contrary, there were cases with recurrent lesions at the 1st recall visit at the same site or close to it.

Discussion

The main etiology of aphthous lesions is still unknown, meaning no definitive treatment option is recommended. The primary aims of routinely performed procedures are to reduce pain and the lesion size, and prevent recurrence. Photobiomodulation is one of the interventions that has been widely analyzed. In our study, PBM yielded better treatment results, with the defocused technique being more efficient in pain relief and decreasing lesion recurrence, and the focused technique resulting in a shorter healing time. Gender, age and the lesion site did not seem to affect the course of the disease or the response to the different treatment modalities ($p > 0.05$). However, the tip of the tongue showed significantly more pain resistance as compared to other sites, while the lip mucosa experienced the least pain duration (95% reliability).

Ahmed et al. performed a systematic review in 2020 to compare the efficacy of PBM and topical interventions used to treat patients with aphthous lesions.[19] Their results were based on 330 patients included in 5 randomized controlled trials.[15,20–23] They reported that PBM was a more effective treatment option than topical medications, such as triamcinolone acetonide, amlexanox, granofurin, and solcoseryl.[19]

The pioneering work published by Pinheiro et al. started a new era in PBM application for the clinical treatment of various disorders.[24] There have been many studies on this topic since then, analyzing the impact of PBM on healing, and showing the approach to be a safe method with limited complications.[25,26] Furthermore, patients treated with this method can drink, eat and brush their teeth a few days after the procedure.[10,27]

The Nd:YAG laser was utilized in the current study. However, the efficacy of other types of lasers in treating patients with intraoral wounds has also been analyzed. Indeed, the treatment protocol may impact the efficacy of PBM. However, there is limited data regarding the optimal treatment approach with Nd:YAG lasers. On the other hand, studies have been performed on other lasers to compare different exposure times and wavelengths. For instance, Rocca et al. analyzed the impact of 4 different wavelengths of PBM (i.e., 2,940 nm, 808 nm, 450 nm, and 635 nm) on treating RAS patients.[28] Their results showed superior results for the 635 nm diode lasers.[28] Similar to the current study, Sharon-Buller et al. reported the successful clinical application of the CO_2 laser in mitigating severe pain in oral aphthosis treatment.[29] The patients manifesting stress-induced, chemoradiotherapy-related and immune-associated oral aphthosis acknowledged prompt pain relief and swift recovery after PBM.[29]

Besides oral lesions, PBM has been applied to overcome other maxillofacial disorders. Pinheiro et al. published a thorough study on PBM for the management of maxillofacial region disorders, including temporomandibular joint (TMJ) pain, trigeminal neuralgia, muscular pain, aphthous lesions, inflammation, post- and preoperative tooth hypersensitivity, and small hemangiomas.[24] Photobiomodulation has been proven to benefit the treatment of various conditions of the maxillofacial region.[30–32]

Recent clinical and laboratory studies have shown that the photobiological and photochemical effects of low-level lasers in injured tissues can stimulate epithelialization, vascularization and the collagen synthesis by fibroblasts. Initially, the stimulation of lymphocyte and granulocyte activity enhances the rate of necrotic tissue removal. Then, fibroblastic activity occurs, which increases the production of collagen. This is followed by the invagination of the capillaries into the regenerated tissues and the promotion of epithelialization, resulting in accelerated tissue regeneration and pain alleviation. Nonetheless, the literature on this topic lacks comparisons between the available therapeutic (biostimulation) and surgical (ablation) approaches.

Conclusions

Therapeutic Nd:YAG lasers, with the conditions and adjustments of the present study, may be successfully applied in RAS lesion management, using either focused or defocused scanning techniques.

Ethics approval and consent to participate

Ethical approval for the study was granted by the ethics committee at Tehran University of Medical Sciences, Iran (ethical code: 3316). 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

Effect of vitamin B17 (amygdalin) found in apricot kernel on the irradiated salivary glands of albino rats

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

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Abstract

Background. Radiotherapy is used as a treatment for head and neck cancers but increases the risk of salivary gland hypofunction. The management strategies include pharmacotherapies such as salivary substitutes and sialagogues which are largely temporary. In this study, we examine the regenerative potential of vitamin B17 to improve salivary gland function.

Objectives. The present investigation aims to identify the effect of vitamin B17 (amygdaline) on the irradiated parotid salivary gland of albino rats.

Material and methods. Twenty-eight adult male albino rats were randomly divided into two groups subjected to irradiation procedure. Fourteen were in the control group, receiving a daily 5 mL saline by oral gavage (7 rats for 14 days and 7 rats for 30 days) while the other fourteen were treated with a daily dose of vitamin B17 (grounded apricot kernel; GAK) at 400 mg/kg in 5 mL of saline by oral gavage (7 rats for 14 days and 7 rats for 30 days). The parotid glands were dissected from the two groups at 14 and 30 days from the day of exposure to irradiation. The parotid gland sections were subjected to H&E stain, immunohistochemical localization of epidermal growth factor (EGF) and PCR using transforming growth factor beta 2 (TGF-β2).

Results. The histological abnormalities corroborate with the immunohistochemical localization of EGF and the PCR results of TGF β2, as their up-regulation in the control group demonstrate oxidative stresses and inflammation. The Treatment with GAK decreased oxidative stress and inflammation while promoting tissue regeneration.

Conclusions. Vitamin B17 is a promising anti-inflammatory agent that boosts immunity, as the experimental group showed better histological architecture of the parotid gland than the other one.

Keywords: irradiation, parotid gland, vitamin B17, amygdalin, ground apricot kernel

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Introduction

Radiation therapy is a double-edged sword, commonly used to treat patients with head and neck squamous cell carcinoma with negative consequences for salivary glands hypo-function.[1] Forty percent of patients exposed to radiotherapy, suffered from induced impairment of salivary gland function and consequent xerostomia (dry mouth), leading to a detrimental impact on oral health and quality of life.[2] Ionizing radiation has direct and indirect effects on macromolecules. Living cells absorb ionizing radiation directly disrupting atomic structures, leading to chemical and biological changes. It causes radiolysis of cellular water, generating reactive chemical species by activating oxidases and nitric oxide synthases. It disturbs mitochondrial functions significantly contributing to persistent alterations in lipids, proteins, nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). Eventually, results in physical and chemical damage to tissues that may lead to cell death or neoplastic transformation. In many cases, ionizing radiation-induced cell death has been identified as apoptosis.[3]

Exposure of salivary glands to radiotherapy leads to a significant loss of acinar cells. However, the mechanism of this cellular attrition is widely debatable. Fibrosis and loss of tissue occur after the loss of function, and the onset and severity of this phase of cellular damage differ among the various species. Even if the fibrosis is not extensive, Loss of salivary fluid secretion still leads to xerostomia and associated complications such as difficulty swallowing, rampant dental caries, oral mucosal lesions and fungal infection.[4]

Thus, the mechanism involved in the irradiation-induced loss of salivary gland function is a subject of great interest in the field, with clinical studies directed towards assessing therapies targeted to the recovery of cell function, prevention of functional loss, or regeneration of salivary glands.[5]

The management strategies include stringent dental and oral hygiene and pharmacotherapies, such as salivary substitutes and sialagogues. Mandelonitrile beta-D-gentiobioside, which is commonly known as vitamin B17, is a natural cyanide-containing substance called nitriloside. Laetrile or amygdaline, the extract form of vitamin B17, is effective in treating cancer as it targets and destroys mutated cells, acts as an anti-inflammatory agent and boosts immunity.[6]

It is mandatory to provide clinically safe and efficient radioprotection agents to scavenge reactive oxygen species (ROS) and reduce the risks of radiotherapy. Antioxidants (AOs) are the primary key of protection against the damage of free radical ions and are essential for maintaining optimum health. Antioxidants have shown potential benefits in preventing or counteracting cellular damage, cancer, ageing, and other diseases.[7]

Epidermal growth factor (EGF) is a transmembrane protein that stimulates cell growth by binding to epidermal growth factor receptor (EGFR) with intrinsic tyrosine kinase activity, to excite target cells. The receptor undergoes dimerization and auto-phosphorylation upon the interaction with EGF-EGFR. It has another role in regulating the expression level of various transcription factors through multiple signaling pathways.[8]

Transforming growth factor beta 2 (TGF-β2) is a multifunctional protein playing an important role in the process of development throughout life, controlling various cellular activities.[9] It is also important in the process of angiogenesis, cell growth, apoptosis, cell migration, cell differentiation, cell-matrix remodeling, epithelial-mesenchymal transition, and wound healing.[10]

Since all the previously mentioned methods are only transient as they do not treat causes of xerostomia, they do not treat the salivary gland dysfunction but only provide symptomatic relief for dry mouth. Therefore, there is an unmet need for new treatment strategies for long-term salivary gland regeneration with minimal adverse effects and greater potency.[11]

Material and methods

The experiment was conducted according to the guidelines of the Care and Use of Laboratory Animals 8th Edition 2011, in the animal facility within the Faculty of Medicine, Cairo University (Approval No: CU/III/S/93/17).

Preparation of the material

Vitamin B17 (amygdaline) was obtained by grinding apricot kernels and administered via gavage to the rats. Amygdaline was given in a dose of 400 mg/kg ground apricot kernel (GAK).[12]

Apricots (Prunus armeniaca L.) were purchased from the local fruit market. Apricot seeds contain approximately 20-80 μmol/g of amygdalin.[13] Apricot flesh was removed from fruits; the apricot outer shell was washed with tap water and air-dried at 30°C for about 2 weeks, then the outer shell of the apricot was cracked manually and the edible part (kernel) was stored at -20°C in sealed plastic bags until used. The apricot kernels were soaked in warm distilled water for 1 h to facilitate the manual removal of the thin layer coat on the kernel. Apricot kernels were ground and dissolved in saline to be given intragastrically to the rats. The GAK was prepared freshly within 1 h before administration.

Experimental animals

Rattus Norvegicus were used in this study since they are genetically similar. Notably, the albino with its red eyes and white fur is an iconic model organism for scientific research in a variety of fields.[14]

Twenty-eight 3-4 month old adult male albino rats weighing approximately 200 g were obtained from the animal facility, Faculty of Medicine, Cairo University. All the rats were subjected to a single dose of gamma-radiation of 5 Gy at the National Center for Radiation Research and Technology (NCRRT), housed by the Atomic Energy Authority, Cairo, Egypt. Irradiation was performed by using Canadian Gamma Cell-40 biological irradiator (137-cesium), manufactured by the Atomic Energy of Canada Limited, Ontario, Canada.

Housing

The rats were allowed to acclimatize for one week before the experiment. They were housed in individual cages and maintained at a temperature of 20-24 C in a 12:12 h light: dark cycle during the experiments and fed with standard food pellets and tap water ad libitum.

Allocation and blinding

The rats were randomly grouped by an online random number generator (random.org). All the histological sections and the histomorphometric analysis were scored by two independent observers blind to the rats' group identities. The scores given by the observers for each section were averaged and used in further analysis.

Study design

The animals were divided into two groups of 14: group A received 5 ml of intragastric saline (no treatment just placebo) daily, immediately after irradiation; and group B received a single intragastric dose of 400 mg/kg (GAK containing vitamin B17) immediately after irradiation.

Half of the rats in each group (7 rats) were sacrificed on day 14 and the other half were sacrificed on day 30. The parotid glands were dissected out, fixed in calcium formalin and embedded in paraffin. Specimen were mounted on glass slides for H&E & immunohistochemical staining. Using an image analyzer, the optical density of immunohistochemical staining of EGF in terms of percent of the total area was calculated by digital image analysis.

Animals were anesthetized with ketamine HCL 50 mg/kg then trans-cardially perfused with 4% formaldehyde. The parotid salivary glands were dissected and collected for histological and histochemical examination.

The flow chart of the study design is presented in Fig. 1.

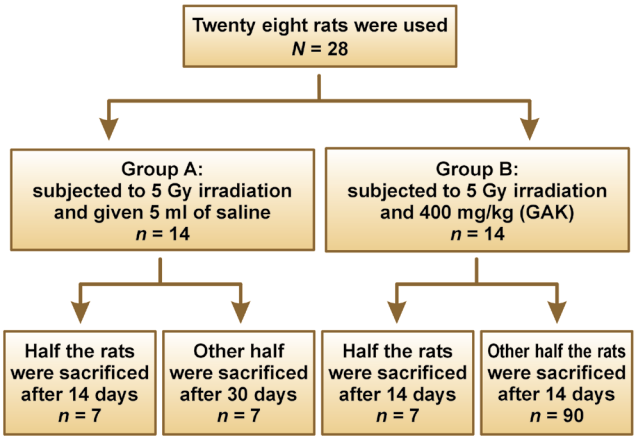

Fig. 1. Flowchart of the study design

Methods of investigation

Histological evaluation

Hematoxylin & eosin stains are commonly employed for histologic and medical studies. Sections were deparaffinized and placed in xylene, hydrated by passing through descending concentrations of alcohol baths and water. 95% alcohol for 2 minutes and 70% alcohol for 2 minutes followed by distilled water. The sections were then immersed in Harris hematoxylin solution for 8 minutes, differentiated in 1% acid alcohol for 30 seconds and washed in running tap water for 1 minute. Hematoxylin was "blued" in 0.2% ammonia water or saturated lithium carbonate solution for 30-60 seconds Followed by another wash under running tap water for 5 minutes and a rinse in 95% alcohol. The sections were counterstained with eosin-phloxine solution for 30-60 seconds, dehydrated through 95% alcohol with 2 changes 5 min absolute alcohol washes. Finally, the sections were cleared in 2 changes 5 min of xylene washes and mounted with xylene based mounting medium. Sections were dehydrated in increasing concentrations of alcohols and cleared in xylene.[15]

Immunohistochemistry

It was used to measure the expression of EGF. Routine immunostaining procedures were apllied to stain formalin-fixed and paraffin-embedded sections.

Five micron-thick sections were used. Specimens were dewaxed in 3 × 5 min xylol washes. The tissue specimen was placed into a glass slide chamber and filled with the processing buffer (citric acid buffer, pH 6.0, preferably pH 6.5-7.0) and incubated at 121°C for 15 min for antigen retrieval. The glass slides were taken out of the chamber, rinsed 3 times with distilled water or PBS by emptying and refilling the chambers 40 min after. Three to four drops of 1:50 primary monoclonal mouse anti-EGF antibodies in PBS were used on the sections for a 20 minute incubation. Endogenous peroxidase activity was blocked by using freshly made 0.3% H_20_2 in methanol for 20 min followed by 3-5 min PBS washes. Incubation with monoclonal antibody, 4°C, overnight. On the next day, the sections were washed with PBS for three 5 min washes and incubated (60-120 min) with the secondary antibody, MAX-PO(MULTI) at room temperature. An-

other three 5 min washes with PBS was performed before applying the diaminobenzidin (filtered (0.01% DAB in 0.5 M Tris/HCI (pH 7.4)) substrate for 10 min at room temperature. H_20_2 was added to a final concentration of 0.01%. At the end of the chromogenic staining, the sections were washed with running tap water for 3 min and counterstained with Mayer's hematoxylin for 30 s. After a brief rinse with tap water, the sections were dehydrated with increasing concentrations of ethanol: 50%, 70%, 96%, absolute for 3 min. each. Specimens were cleared with xylol with 3 × 3 min washes and mounted with mounting medium.[16]

Histomorphometric analysis of immunohistochemical staining

The immunostained sections were examined using an image analyzer computer system to measure the optical density in percent area. Immunoreactivity of EGF was performed through the images analysis using a computer (Leica Quin 500 Microsystems, Switzerland) consisting of color video camera connected to the microscope for image acquisition through a PC. The intensity of the reaction within the cells was determined by measuring the optical density in 5 small sampling fields in each section under 400x. The areas showing EGF positive brown immune staining were chosen for evaluation, regardless of the staining intensity. These areas were masked by a blue binary color to be measured by the computer system. The image analyzer is calibrated automatically to convert the measurement units produced by the image analyzer program into actual micrometer units. Mean values and standard deviations were calculated for each specimen.

Quantitative real-time PCR

It was used to measure the expression of TGF-β2.

Total RNA was isolated using Qiagen tissue extraction kit (Qiagen, USA) according to the instructions of the manufacturer. The total RNA (0.5–2 μg) was used for cDNA conversion using high capacity cDNA reverse transcription kit Fermentas, USA). Real-time qPCR amplification and analysis were performed using an Applied Biosystem software version 3.1 (StepOne™, USA). The qPCR assay with the primer sets was optimized at the annealing temperature. The relative quantitation was calculated according to Applied Biosystem software.

Statistical analysis

ANOVA was used to compare between all conditions. Data are presented as the mean ± standard deviation (SD). A p-value of $p < 0.001$ was considered to be statistically significant. This was followed by Tukey's post hoc test when ANOVA indicated a significant difference.

Results

Histological examination

The H&E stained sections of group A (irradiated only) showed degenerative changes at both day 14 and day 30. Acinar shrinkage, multiple cytoplasmic vacuoles and nuclear anisonucleosis and poikilonucleosis were detected. The duct system showed thinning of the epithelial lining of some excretory ducts and stagnant secretion was found in others. Both intercalated and striated ducts showed no detectable changes. Areas of fibrosis were detected in the connective tissue that was chronically infiltrated by inflammatory cells. Additionally, the blood vessels suffered from dilatation and some appeared engorged with RBCs. Also, some blood vessels showed hyalinization and others were ruptured (Fig.2,3).

On the contrary, the H&E stained sections of group B (irradiated group receiving treatment) on day 14 showed improved degenerative changes that further improved at

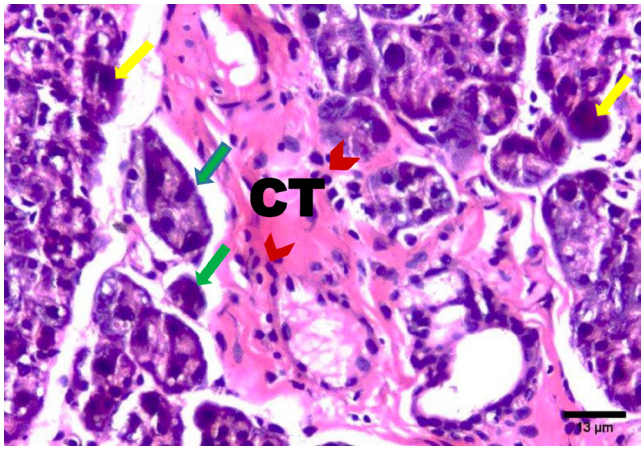

Fig. 2. Photomicrograph of rat parotid gland of group A (day 14) showing: shrunken acini (green arrows), acini showing clumping (yellow arrows), fibrosis in connective tissue (CT), inflammatory cells (red arrow heads), excretory duct showing signs of degeneration (red arrow) (H&E, Orig. Mag. x400)

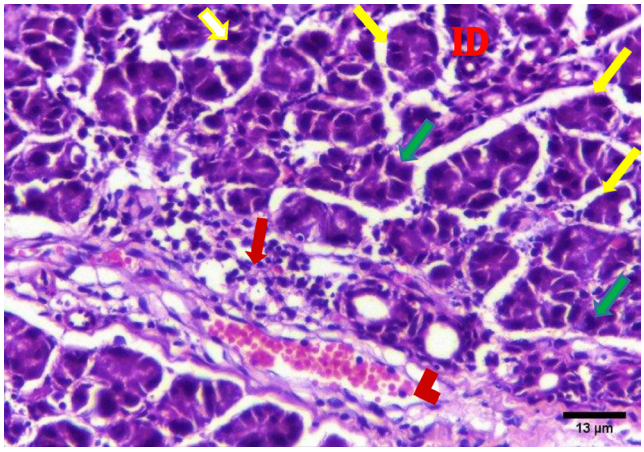

Fig. 3. Photomicrograph of rat parotid gland of group A (day 30) showing: shrunken acini (yellow arrows), clumped acini (white arrow), dilated blood vessels engorged with RBCs (red arrow head), nuclear anisonucleosis & poikilonucleosis (green arrows), inflammatory cells within connective tissue (red arrow), intercalated duct seems to be normal (ID) (H&E, Orig. Mag. x400)

day 30. The positive outcome is demonstrated by decreased acinar shrinkage and cytoplasmic vacuolization in some acini, others suffered from degeneration in a few areas, also nuclear anisonucleosis and poikilonucleosis were less pronounced in this group. Mitotic figures were observed within some acinar cells. The striated and intercalated ducts maintained their form and remained intact. Few excretory ducts showed areas of thinning in their epithelial lining. The connective tissue appeared with fewer areas of fibrosis and few chronic inflammatory cells infiltrated. Few blood vessels were dilated and engorged with RBCs (Fig. 4,5).

Immunohistochemical examination

Immunohistochemical investigation of parotid glands of group A on day 14 revealed strong cytoplasmic, membranous and nuclear expression of EGF in both acini and ducts. There was no staining in the connective tissue. Immunostained specimens from the same group on day 30 showed strong membranous, cytoplasmic and nuclear expression of the EGF in acinar & ductal cells. Few scattered serous cells showed no nuclear expression. The connective tissue showed no expression (Fig. 6,7).

Immunohistochemical investigation of parotid glands of group B on day 14 showed weak membranous, moderate cytoplasmic and nuclear reactivity of acinar cells. Some acinar cells showed no nuclear expression. The ductal cells showed strong membranous, cytoplasmic and nuclear expression. There was no immunoreactivity in the connective tissue. Immunostained sections from the same group on day 30 showed weak membranous, cytoplasmic and negative nuclear expression in serous cells. The duct system revealed strong membranous, cytoplasmic and nuclear expression. The connective tissue did not stain for EGF (Fig. 8,9).

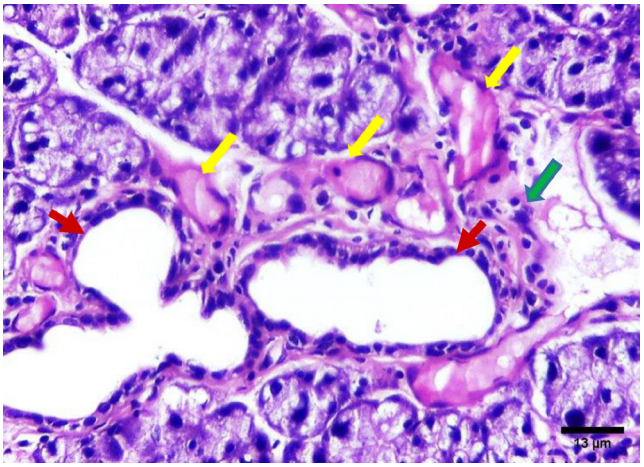

Fig. 4. Photomicrograph of a rat parotid gland of group B (day 14) showing: hyalinized blood vessels with discontinuity in their linings (yellow arrows), excretory duct showing thinning in their lining (red arrows), inflammatory cells infiltration (green arrow) (H&E, Orig. Mag. x400)

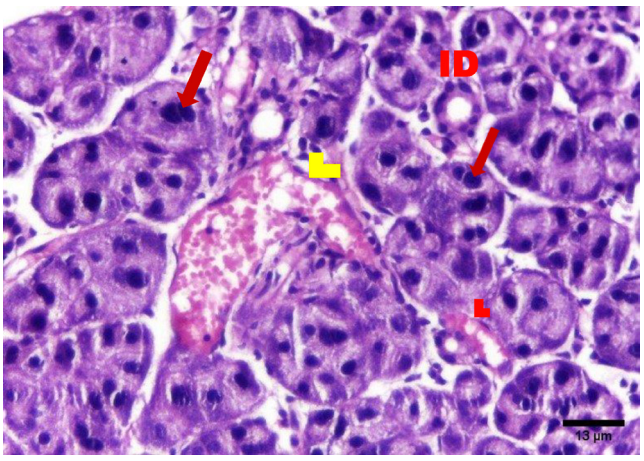

Fig. 5. Photomicrograph of a rat parotid gland of group B (day 30) showing: mitotic figures within acinar cells (red arrows), dilated blood vessels engorged with RBCs (yellow arrow head), hyalinized blood vessel (red arrow head), normally appearing intercalated ducts (ID) (H&E, Orig. Mag. x400)

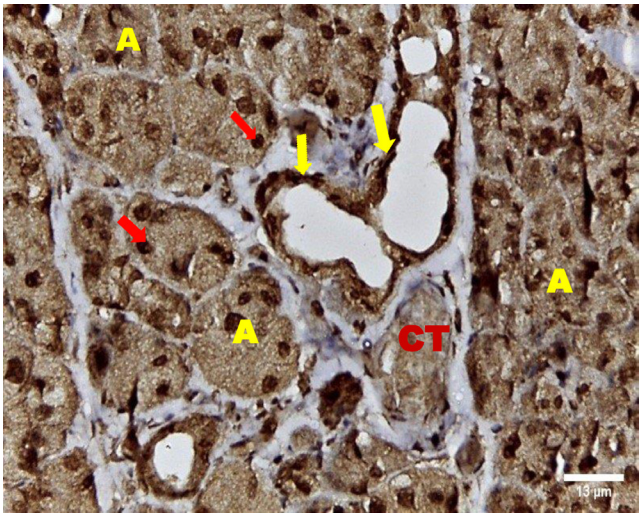

Fig. 6. Photomicrograph of a rat parotid gland of group A (day 14) showing immunoreactivity of EGF: strong membranous, cytoplasmic (A) and nuclear (red arrows) expression within serous acini, excretory duct showing strong membranous, cytoplasmic and nuclear expression (yellow arrows), and negative expression within the connective tissue (CT) (DAB, Orig. Mag. x400)

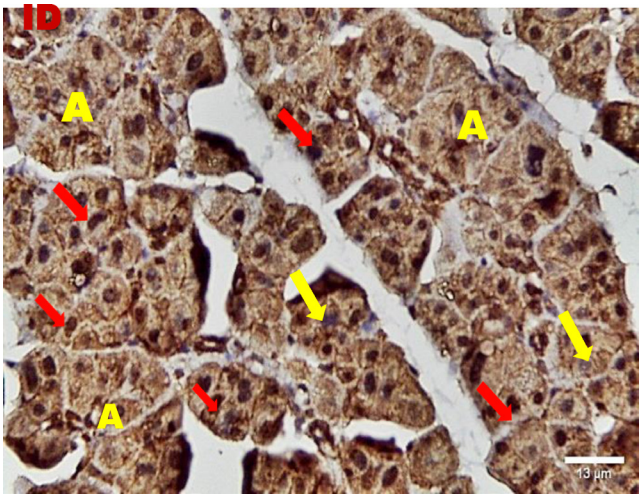

Fig. 7. Photomicrograph of a rat parotid gland of group A (day30) showing immunoreactivity of EGF: strong membranous, cytoplasmic (A), and nuclear (red arrows), acinar cells with negative nuclear expression (yellow arrows), strong membranous, cytoplasmic & nuclear expression within intercalated duct (ID) (DAB, Orig. Mag. x400)

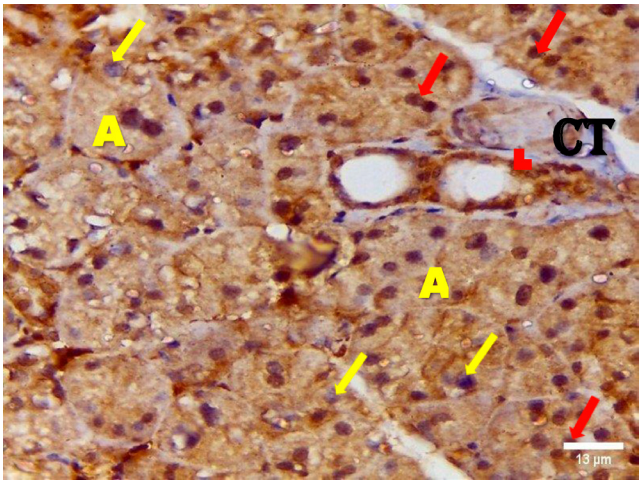

Fig. 8. Photomicrograph of a rat parotid gland of group B (day14) showing immunoreactivity of EGF: weak membranous, moderate cytoplasmic (A), moderate nuclear (red arrows), negative nuclear (yellow arrows), expression within serous acini, excretory duct (red arrow head) showing strong membranous, cytoplasmic and nuclear expression, while connective tissue shows negative expression (CT) (DAB, Orig. Mag. x400)

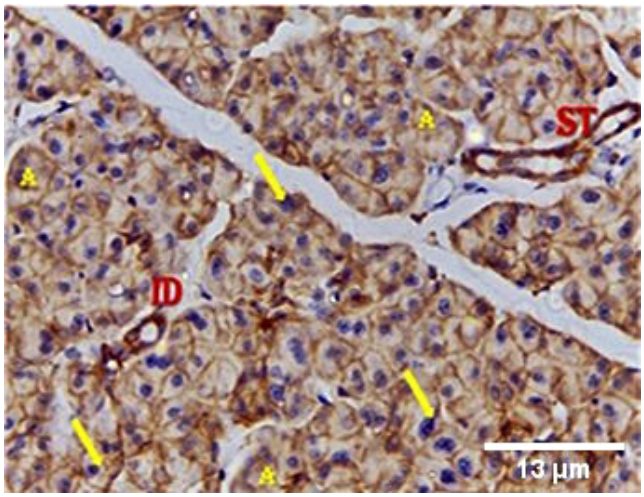

Fig. 9. Photomicrograph of a rat parotid gland of group B (day 30) showing immunoreactivity of EGF: weak membranous and cytoplasmic (A), negative nuclear expression (yellow arrows) within serous acini, intercalated duct (ID) and striated duct (ST) showing strong membranous, cytoplasmic and nuclear expression (DAB, Orig. Mag. x400)

Statistical analysis

Immunohistochemical analysis was quantified using percent area. The analysis suggests the mean EGF expression covered more tissue area in group A compared to group B. ANOVA test revealed that the difference was statistically significant (P<0.0001) between all the studied groups. Tukey's post hoc test revealed no significant difference between group B at 14 days and group A at 30 days. Moreover, no significant difference could be observed between group B at 14 and 30 days (Table 1, Fig. 10).

PCR results of TGF-β 2 revealed that the greatest mean value was detected in group A while the lowest mean value was recorded in group B. ANOVA test revealed that the difference was statistically significant (P<0.0001) between all

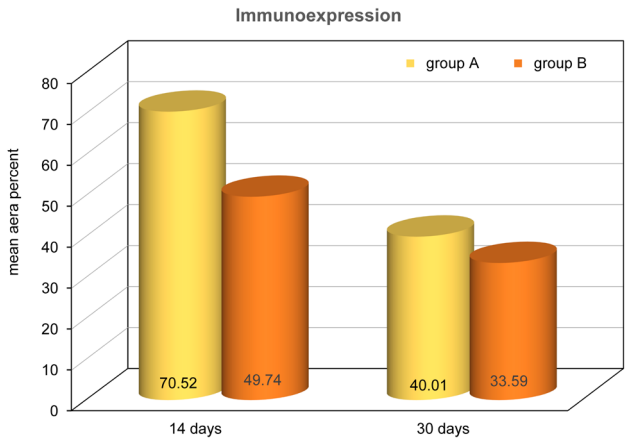

Fig. 10. Column chart showing mean area percent of EGF immunoreactivity in Group A and Group B at each date

Table 1. Area percentage of immunoreactivity of epidermal growth factor (EDF) in different groups (ANOVA)

Group	Time point	*M* ±*SD*	*SE*	95% *CI*		min	max	F	*p*-value
				lower bound	upper bound				
Group A	day 14	70.52 ±12.73[a]	5.69	54.71	86.33	58.43	89.78	18.25	<0.0001*
	day 30	49.74 ±5.03[b]	2.25	43.49	55.99	43.42	57.50		
Group B	day 14	40.01 ±8.38[b,c]	3.75	29.60	50.41	26.98	49.92		
	day 30	33.59 ±5.26[c]	2.35	27.05	40.12	25.35	39.12		

* statistically significant; values with different superscript letters are significantly different (Tukey's post hoc test).

Table 2. Mean values of transforming growth factor beta 2 (TGF-β2).in different groups (ANOVA)

Group	Time point	*M* ±*SD*	*SE*	95% *CI*		min	max	F	*p*-value
				lower bound	upper bound				
Group A	day 14	2.92 ±0.13[a]	0.06	2.76	3.08	2.73	3.06	188.49	<0.0001*
	day 30	2.37 ±0.26[b]	0.11	2.05	2.69	2.10	2.66		
Group B	day 14	1.23 ±0.05[c]	0.02	1.17	1.30	1.15	1.30		
	day 30	0.87 ±0.11[d]	0.05	0.74	1.00	0.74	0.99		

* statistically significant; values with different superscript letters are significantly different (Tukey's post hoc test).

studied groups. This was followed by Tukey's post hoc test when ANOVA indicated a significant difference. Tukey's post hoc test revealed a statistically significant difference between the two subgroups (Table 2, Fig. 11).

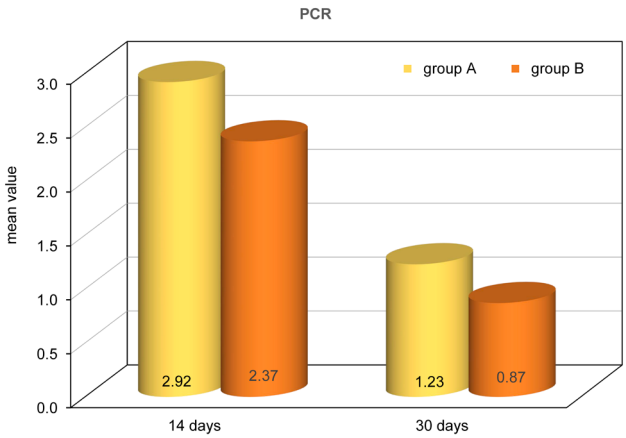

Fig. 11. Column chart showing mean values of TGF-β2 in group A and group B at each date

Discussion

Acinar cells in the salivary gland are destroyed during radiation treatment for head and neck cancer that results in a lifetime of hyposalivation and co-morbidities. [17] In the present investigation, a sub-lethal dose of radiation at 5 Gy was used. [18] The chosen radiation dose was selected similar to Avila who used the same ionizing radiation dose and reported radiation-induced changes in the parotid gland of rats.[5]

The results of the present study showed acinar degeneration, shrinkage and cytoplasmic vacuolization in group A (day 14 and day 30). Boraks, Tampelini, Pereira, and Chopard have linked acinar degeneration and vacuolization to the expansion of the endoplasmic reticulum which is associated with the cellular status that precedes apoptosis this expansion is related to compression of the nuclear material.[19]

In the current investigation, shrinkage of some serous acini, fewer areas of acinar degeneration and fewer cytoplasmic vacuoles were observed within some serous acini in group B which received vitamin B17 (day 14 and day 30). The changes on day 30 were less than that on day 14. These changes were similar to findings reported by Qadir and Fatima where amygdalin acts as an antioxidant and a scavenger of the harmful and highly hydroxyl radicals by maintaining a healthy body pH.[20]

Moreover, the observed nuclear changes (anisonucleosis & poikilonucleosis) and clumped acini with condensed chromatin reported in this work are in agreement with Krishnan et al. who observed shrunken nuclei with condensed chromatin in irradiated salivary gland acinar cells. The author assumed that the irradiation dose was not high enough to cause complete DNA destruction and complete nuclear disintegration.[21]

Intercalated and striated ducts showed no detectable changes in our study in group A (day 14 and day 30) and group B (day 14 and day 30). This could be attributed to the powerful regenerative properties of the ductal structure of the salivary gland.[22]

In the present investigation, excretory ducts showed degeneration of their lining, others showed stagnant secretion in group A (after 14 days). These findings corroborate with those reported by Kassab, and Tawfik who detected degeneration of excretory duct and stagnant secretion due to oxidative stresses and inflammation in albino rats subjected to long term use of caffeinated drinks.[23]

In this study, the stagnant secretion found in some excretory ducts in group A was previously shown by Halawa, Mohamed, and Obeid who found some dilated excretory duct with stagnant secretion and concluded that the mitochondrion is the most vulnerable cell organelle to toxic agents and oxidative stresses. Upon mitochondrial destruction in excretory ducts, cellular metabolism was affected with advanced cellular destruction. Destructed mitochondria result in adenosine triphosphate ATP consumption and subsequently, impaired exocytosis took place, so no energy for secretion causing stagnant secretion and ductal dilatation.[24]

In the current work, connective tissue fibrosis was noted mainly in group A (day 14 and day 30), consistent with Huang, Chen, and Miao who demonstrated that the connective tissue stroma of parotid and submandibular gland of rats undergoes adiposis and fibrosis after irradiation.[25]

Milazzo et al. reported that vitamin B17 which is a main component of GAK has many potentialities in addition to its protective effect against cancer. It boosts immunity and eliminates harmful cells as it acts as an antioxidant. The protective properties could account for the decreased fibrosis in group B (day 14 & day 30) in this study.[6] This interpretation is supported by Abdel-Rahman who carried out a study on rats suffering from induced hepatic fibrosis and reported that the addition of GAK to rats' diet inhibited acute hepatocellular injury and fibrosis.[12]

The presence of blood vessels engorged with red blood cells was observed in this investigation in group A (14 and 30 days) which decreased in group B (14 & 30 days). These findings were in agreement with the results of Redman who found thinning and discontinuity in the endothelium lining of blood vessels of the parotid salivary gland of rats that was subjected to irradiation, leading to a compromised blood supply.[26]

Chronic inflammatory cells infiltration in the connective tissue in this study were clearly observed in group A (day 14 & day 30), replicating findings by Limesand, Said, and Anderson, who found chronic inflammatory cells in response to irradiation of parotid salivary glands of rats.[27]

Chronic inflammatory cells and dilated blood vessels decreased in the treatment group at 14 days with continued improvements at day 30 compared to controls., These observations may be mediated by the anti-inflammatory properties of GAK, where Minaiyan, Ghannadi, Asadi, Etemad, and Mahzouni found GAK treatment in treating colon inflammation in rats also resulted in a decrease in inflammatory cells.[28]

Mitotic figures were observed in group B specifically on day 30. This finding was explained by Mohamed, El-Sakhawy, Sheriff, and Shredah, who assumed that mononuclear cells duplicate DNA while undergoing endomitosis. Therepeated prevalence of mitotic figures can be explained by being an adaptive trial of the acinar cells to heal after injury.[29]

Noticeably, the immunohistochemical results in the current study supported the histological results - strong cytoplasmic, membranous and nuclear EGF expression of acini and ducts in group A on day 14 and day 30 was observed. These results were in agreement with Shang et al. who demonstrated upregulation of EGF in most mucoepidermoid carcinomas, which surges to 80% in case of lymph node metastases.[30]

The results of this study demonstrated strong nuclear expression within the acini of group A at day 14 and day 30, which was clarified by Dittmann et al. who concluded that upon cell irradiation EGF-EGFR complex are translocated to the cell nucleus, principally in regions with uncoiled chromatin (euchromatin) because these areas are easily accessible for DNA repair processes after exposure to irradiation.[31]

Moreover, Vuorinen, Rajala, Ihalainen, and Kallioniemi found that the complex is linked to co-transport of some associated proteins such as Ku70 and Ku80 as well as protein phosphatase 1 which is linked to the nucleus and is involved in the regulation of DNA-protein kinase. Nuclear import of the EGFR-linked complex is permitted by a karyopherin-dependent process which plays an important role in the process of regeneration.[32]

Group B in the current study demonstrated weak membranous, moderate cytoplasmic and nuclear reactivity on day 14, while on day 30 it showed weak membranous, cytoplasmic and no nuclear expression of EGF. These results were explained by Dittmann et al. who reported that the use of radical scavengers eliminated radiation-induced EGF nuclear translocation.[31] Moreover, the connective tissue of all groups showed no EGF expression, which is in agreement with Zhuang, and Liu who assumed that EGF and EGFR are located mainly in epithelial cells not in the connective tissue under normal or pathologic conditions.[33]

PCR results of this study are consistent with the histological and immunohistochemical results as they revealed an increase in the amount of TGF-β 2 in group A on day 14 and day 30, while this amount decreased in group B on day 14 and day 30, respectively.

The increase in TGF-β 2 levels in group A have been reported by Barrientos, Stojadinovic, Golinko, Brem, and Tomic, who suggested the involvement of TGF-β2 in granulation tissue formation, through stimulation of angiogenesis, fibroblast proliferation, myofibroblast differentiation, and matrix deposition.[34]

To the best of our knowledge, little or sparse documents were available on how ground apricot kernel (vitamin B17) affected irradiated parotid salivary glands, neither its potential effect on TGF-β2 levels nor EGF expression.

Conclusions

A single dose of whole-body 5 Gy irradiation-induced degenerative changes of the parotid glands of albino rats. Administration of vitamin B17 (GAK) intragastric to the irradiated rats improved the histological features of the irradiated salivary gland. The histological results and the observed immunohistochemical localization of EGF and PCR results of TGF β2 support the translational therapeutic potential of GAK on salivary gland dysfunction associated with oral cancers by downregulating oxidative damage and inflammatory responses.

Ethics approval and consent to participate

The experiment was conducted according to the "Guide for the Care and Use of Laboratory Animals" 8th ed., 2011, in the animal facility within the Faculty of Medicine, Cairo University, Egypt (approval No. CU/III/S/93/17).

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

Inhibitory effect of silver nanoparticles synthesized using the chamomile extract against *Streptococcus mutans* cariogenic pathogen

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

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Abstract

Background. With the recent use and development of nanomaterials, silver nanoparticles (AgNPs) are gaining much attention as a promising antibacterial agent for use in caries prevention.

Objectives. This study aimed to biosynthesize AgNPs using chamomile extract as a reducing agent and to investigate its inhibitory effect against *Streptococcus mutans* (*S. mutans*) dental bacteria.

Material and methods. Chamomile extract was prepared by sonication and added dropwise to silver nitrate (1mM) solution to synthesize AgNPs. Its formation was confirmed spectrophotometrically, and its size was determined. The disc diffusion method was used to test the antibacterial activity of the biosynthesized AgNPs against *S. mutans*. Also, its minimum inhibitory concentration (MIC) was assessed.

Results. The spectrum of biosynthesized AgNPs showed a maximum peak at 454 nm, and the peak area increased with increasing time. The mean AgNP size was 41 nm. The inhibition zone diameter recorded for AgNPs against *S. mutans* was 10 mm, while the MIC was 280 µg/ml.

Conclusions. AgNPs biosynthesized using chamomile extract were proven to exert good antibacterial activity against cariogenic S. mutans. Using chamomile extract as a reducing agent can provide a rapid, affordable, and eco-friendly approach for AgNP production, which could be incorporated into various dental vehicles for dental caries prevention.

Keywords: silver nanoparticles, Streptococcus mutans, chamomile, antibacterial, dental caries

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Introduction

Dental caries is the most common oral disease in childhood. The World Health Organization (WHO) reported that dental caries affects 60-90% of school-aged children, negatively affecting the quality of life of children and their families. Caries can frequently result in extreme discomfort and growth retardation and have negative effects on body weight and height in children.[1,2]

Dental caries is a multifactorial, dynamic, biofilm-mediated, sugar-driven disease that causes demineralization of the dental hard tissues.[3]

Streptococcus mutans (S. mutans) are Gram-positive bacteria commonly found in human dental plaques. *S. mutans* is the principal bacteria involved in the onset of dental caries because of its acidogenic and aciduric properties, which lead to colonization of the tooth surface, production of dental plaque, and demineralization of hard dental tissue.[4, 5] Since its discovery as the causative agent of dental caries, this bacterium has been studied as a potential target for disease prevention through antimicrobial drugs and the development of a vaccine.[6]

Silver has been used as an antimicrobial agent for over a century due to its wide range, low toxicity, and absence of bacterial resistance. Silver nitrate is one of the most common silver salts exhibiting antimicrobial properties and has been widely used as a caries preventive agent for permanent molars, a cavity sterilizing agent, and a dentine desensitizer to reduce the occurrence of caries in deciduous dentition.[7]

Nanotechnology is viewed as a pioneering and realistic research field due to the growing desire for advances in diagnosis and treatment approaches.[8]

Using silver nanoparticles (AgNPs) to prevent and treat dental caries by inhibiting biofilm development and regulating the demineralization and remineralization balance is a promising approach for tooth decay prevention and treatment.[9]

AgNPs have 25 times greater antibacterial activity than chlorhexidine and have antiviral and antifungal activity. In different preparations, studies have indicated their use shows promising results for treating early dental caries.[10] Moreover, adhesive systems and composite resins can benefit from the incorporation of AgNPs, as they can prevent secondary caries by exerting significant antibacterial effects at low concentrations.[11]

Many years ago, various chemical and physical technologies, such as laser ablation, lithography, chemical vapor deposition, sol-gel technology, and electro-deposition, were used to synthesize nanoparticles (NPs). However, these approaches are expensive and have also been documented to yield substances that are potentially dangerous to individuals and the environment.[12,13]

The use of plant extract in the synthesis of NPs is particularly popular and is gaining much attention globally. Indeed, the plant-based synthesis of NPs is simple, inexpensive, environmentally benign, and less harmful for human therapy.[14–16]

Chamomile is one of the most widely used medicinal plants (*Matricaria recutita* L.). The species is native to Europe and Asia but is found almost worldwide.[17] More than 8000 tons of chamomile raw material are collected annually globally, with the primary producers being Argentina, Egypt, Poland, and Hungary.[18]

Chamomile has been recognized as an anti-inflammatory, antibacterial, and wound-healing promoter. It decreases plaque development and boosts gingival health with other herbal ingredients such as mouthwash or dentifrice.[19,20]

Several *in vitro* studies evaluated the antimicrobial activity of plant-mediated AgNPs against oral pathogens. AgNPs derived from the leaves of Justicia glauca demonstrated antimicrobial activity against *S. mutans, Staphylococcus aureus (S. aureus), and Lactobacillus acidophilus (L. acidophilus)*.[21] Additionally, plant extracts of Azadirachta indica, Ficus bengalensis, and Salvadora persica demonstrated antibacterial activity against *L. acidophilus, Lactococcus lactis (L. lactis), and S. mutans*.[22] In another study, the biogenic AgNPs derived from Gum Arabic showed antimicrobial activity against *S. mutans*.[23]

In light of combining the therapeutic efficiency of AgNPs and the medicinal potential of chamomile extract for promising use in the prevention of dental caries, the purpose of this study was to biosynthesize AgNPs using chamomile extract as a reducing agent and to investigate the inhibitory effect of biosynthesized NPs against *S. mutans* dental bacteria.

Material and methods

Plant material and extract preparation

Chamomile flower extract solution was prepared by weighing 5 g of chamomile flowers in 50 ml of deionized water, and the mixture was sonicated for two hours at 30°C using an ultrasonic bath at 40000Hz. The extract was then stored in the refrigerator until use.[24]

Preparation and characterization of AgNPs

For the preparation of the AgNP solution, 5 ml of the chamomile extract was added dropwise to 50 ml of silver nitrate (1mM) while stirring using a hot plate and magnetic stirrer set at 50°C for 30 minutes.[25] Silver nitrate used in the experiment was obtained from Sigma-Aldrich. A laser diffractometer, the Zeta Sizer Nano-series, calculated particle size distribution (Nano ZS).[26]

In vitro antibacterial properties of AgNPs

Kirby–Bauer disk diffusion method (determination of the zone of inhibition)

Bauer and Kirby's disk diffusion mechanism calculated the antibacterial activity of AgNPs (0.05mg/ml) and chamomile extract (100mg/ml). In this experiment, paper filter discs (1 cm) were soaked in an AgNP solution. Every bacterium's 0.1 ml culture suspension, modified to 10^8 CFU /ml, was separately seeded into Muller-Hinton agar medium (Merck) and then poured into Petri dishes. At the center of each of these plates, one paper disc from each treatment was aseptically placed, and plates were incubated for 24 hours at 37°C. For each treatment, three duplicate plates were used. Bacterial growth inhibition zones were measured around each disc and recorded in millimeters.[27] Negative control discs were prepared using dimethyl sulfoxide (DMSO), while positive control discs used Ampicillin as a standard antimicrobial agent. The experiments were repeated three times, and the mean data were presented.

Serial dilution method (determination of the minimum inhibitory concentration (MIC))

To assess the antibacterial potential of the biosynthesized AgNPs, MIC was estimated. A series of AgNP concentrations were used to estimate the MIC values and confirm their antibacterial potentials against *S. mutans* ATCC 25175 using the agar dilution method. *S. mutans* stationary phase culture was prepared at 37 ° C and used to inoculate a fresh 5 ml culture to an OD_{600} of 0.05. The 5 ml culture was then incubated at 37 ° C until an OD_{600} of 0.1 was obtained, from which standardized suspensions of bacteria were prepared for a final cell density of $6x10^5$ colony-forming units (CFU)/ml. Different amounts from the tested samples (0-640µl/l) were prepared and combined with 5.0 ml of the standardized bacterial suspension from the measured sample, then applied to the plates and incubated at 37°C for 24 hours. For each sample concentration, CFU were counted and compared with the growth of untreated controls.[28]

Results

AgNP formation and characterization

The formation of AgNPs was observed visually as the solution color changed from a clear to a light brown color (Fig. 1). This color change was the first evidence of silver phyto-reduction and AgNP formation.[29,30]

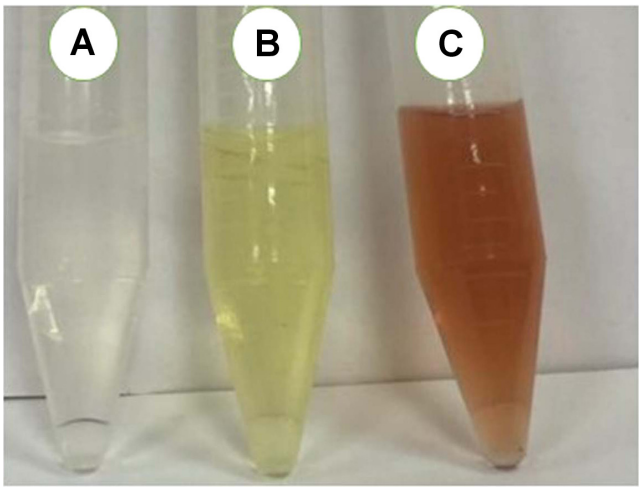

Fig. 1. Formation and characterization of silver nanoparticles (AgNPs)
A – silver nitrate (AgNO3) solution; B – chamomile extract; C – biosynthesized AgNPs.

In the present work, the ultraviolet (UV)-visible spectrum of AgNP biosynthesis using chamomile extract (Fig. 2A) showed a maximum peak at 454 nm, corresponding to the plasmon absorbance of AgNPs.

The stability of biosynthesized AgNPs was observed over 48 hours (Fig. 2B), and the spectra showed that the peak area increased with increasing time.

The average size of the biosynthesized AgNPs was 41 nm. The quality of the biosynthesized AgNPs was confirmed by the single peak obtained (Fig. 3). The data also revealed that the AgNPs biosynthesized using chamomile extract have hydrodynamic diameters, *Z*-average (nm) 76.50, and a polydispersity index value of 0.205.

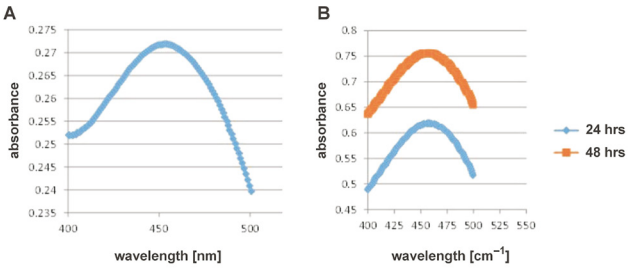

Fig. 2. Spectrophotometric spectra of silver nanoparticles (AgNPs)
A – directly after formation; B – after 24 h and 48 h.

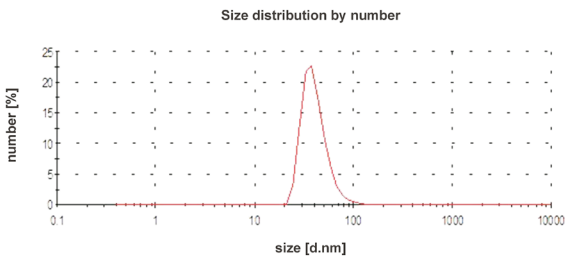

Fig. 3. Particle size distribution of silver nanoparticles (AgNPs)

Antibacterial activity of AgNPs against *S. mutans*

In the present study, the AgNPs biosynthesized using chamomile extract exhibited moderate antimicrobial activity against *S. mutans*, as shown by the inhibition zone's diameter. The mean inhibition zone diameter recorded for AgNPs against *S. mutans* was 10 mm (Table 1).

Minimum inhibitory concentration of AgNPs against *S. mutans*

In the present work, the MIC of biosynthesized AgNPs against *S. mutans* was 56 ±4.1 µg/ml (Table 1).

Table 1. Antibacterial activity and minimum inhibitory concentration (MIC) of silver nanoparticles (AgNPs) and the chamomile extract against *Streptococcus mutans* (*S. mutans*)

Preparation	Inhibition zone [mm]	MIC [µg/mL]
DMSO	none	none
Ampicillin	16.0 ±1.5	24.3 ±2.3
AgNPs	10.0 ±1.0	56.0 ±4.1
Chamomile extract	none	none

DMSO – dimethyl sulfoxide.

Discussion

Eliminating the *S. mutans* bacterial load from the oral cavity is one of the most critical biological targets for dental caries prevention.[31]

Several studies evaluated the antimicrobial effect of nanosilver on *S. mutans* and showed that it had a high impact at low concentrations.[32–34] Nevertheless, the antimicrobial effect of biologically synthesized AgNPs using chamomile extract against *S. mutans* dental bacteria has not yet been investigated. Thus, the present study aimed to biosynthesize AgNPs using chamomile extract as a reducing agent and research the inhibitory effect of biosynthesized NPs against *S. mutans* dental bacteria.

Green syntheses using biological molecules obtained from plant sources in the form of extracts display superiority over chemical and/or natural methods. These plant-based biological molecules go through a highly controlled assembly to make them suitable for metal NP synthesis.[35] Since it is anti-inflammatory, antibacterial, widely distributed, inexpensive, easily attainable, and safe to handle, chamomile extract was chosen as a reducing agent for AgNP synthesis in this study.[36]

Fortunately, the formation of biosynthesized AgNPs was successfully achieved using chamomile extract as a reducing agent, which was monitored visually by the formation of a brown color in the solution. Following previous studies, the appearance of a yellowish-brown color that increased in strength during incubation indicates AgNP formation. This color change occurs due to the excitation of surface plasmon vibrations in the NPs.[30,37]

The biosynthesis of AgNPs was further confirmed by UV-visible spectroscopy, one of the most important and simplest approaches for ensuring NP formation. A maximum spectrophotometric absorbance of 400–460 nm is usually indicative of AgNP formation.[38] As previously reported, the spectrum of UV-vis absorption, the size, and the form of AgNP are well-known to have a very close connection.[30] Therefore, the existence of a single peak in the spectrum demonstrates spherical NP biosynthesis. Furthermore, the spectrum showed no other peaks, meaning that AgNPs were the only particles formed in the solution.[39]

In the spectrum shown in Fig. 2B, the increase in the absorbance peak area with the increase in reaction time may be explained by the reduction of biomolecular silver ions.[29] Based on the particle size distribution results seen in Fig. 3, it is generally agreed that when the polydispersity index (an estimate of the distribution of the population of NPs) shows higher values, this usually means a broad size distribution of several AgNPs.[40]

To analyze or screen the *in vitro* antibacterial activity of an extract of a pure chemical, a variety of laboratory procedures can be applied. The disk-diffusion and broth or agar dilution procedures are the most well-known and basic approaches.[41] In the present study, the disk diffusion method was used because it is a simple and sensitive method that provides categorical results that can be easily comprehended by clinicians.[42]

Up until now, AgNPs' precise antimicrobial mechanism of action is uncertain. However, investigations have revealed that being nanoscale in size, AgNPs can easily penetrate the microbial cell wall/cell membranes via sulfur-containing proteins or thiol groups, damaging the microbial deoxyribonucleic acid (DNA) and eventually causing cell death.[43,44]

In this study, the biosynthesized AgNPs showed an inhibition zone diameter of 10 mm and a MIC of 56µg/ml. This inhibitory effect follows comparable studies in which AgNPs were biosynthesized from natural products.[25,45–47] Several previous studies have reported higher MIC values for AgNP inhibition against *S. mutans* strains, e.g., 60 ± 22.36 µg/ml,[48] 625µg/ml,[49] 50 µg/mL, and 200 µg/mL.[50]

Based on the findings of the present work, the AgNPs biologically synthesized using an aqueous extract of chamomile flowers appear to be a potential and effective bactericidal agent against *S.mutans* that might be used to prevent caries.

Conclusions

The current research has shown that the AgNPs biosynthesized using an aqueous extract of chamomile flowers have good antibacterial activity against the cariogenic

pathogen *S. mutans*. This method can help in the quick, cost-effective, and environmentally reliable synthesis of AgNPs, which can be added to various dental vehicles such as mouthwashes or toothpaste to prevent dental caries. In dentistry, there are limited commercially available products with AgNPs in their composition. Therefore, future studies should be carried out to provide additional dental vehicles containing biosynthesized AgNPs using chamomile flower extract. Their toxicity should also be evaluated before being used as human products. Moreover, well-designed clinical trials are needed to assess the effectiveness of such products in preventing dental caries.

Ethics approval and consent to participate

Not applicable.

Data availability

All data generated and/or analyzed during this study are included in the article.

Consent for publication

Not applicable.

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Original paper

Epitope-based vaccine design against the membrane and nucleocapsid proteins of SARS-CoV-2

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Abstract

Background. The high prevalence and mortality rate of coronavirus disease 2019 (COVID-19) is a major global concern. Bioinformatics approaches have helped to develop new strategies to combat infectious agents, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Indeed, the structural proteins of microorganisms provide suitable epitopes for the development of vaccines to prevent infectious diseases.

Objectives. The present study aimed to use bioinformatics tools to find peptides from the membrane (M) and nucleocapsid (N) proteins with effective cellular and humoral immunogenicity.

Material and methods. Sequences of the M and N proteins were sourced from the National Center for Biotechnology Information (NCBI). The conserved regions of the proteins with the highest immunogenicity were identified and assessed using different servers, and the physicochemical and biochemical properties of the epitopes were evaluated. Finally, allergenicity, antigenicity and docking to human leukocyte antigen (HLA) were investigated.

Results. The data indicated that the best epitopes were LVIGFLFLT and LFLTWICLL (as membrane epitopes), and KLDDKDPNFKDQ (as a nucleocapsid epitope), with significant immunogenicity and no evidence of allergenicity. The 3 epitopes are stable peptides that can interact with HLA to induce strong immune responses.

Conclusions. The findings indicate that 3 common epitopes could effectively elicit an immune response against the disease. Hence, in vitro and in vivo studies are recommended to confirm the theoretical information.

Keywords: HLA, COVID-19, SARS-CoV-2, bioinformatics, multi-epitope vaccine

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Introduction

Coronavirinae is a subfamily of *Coronaviridae*, and it includes 4 genera – alpha, beta, gamma, and delta coronaviruses. Human coronaviruses were first identified in 1965; they are responsible for respiratory tract infections in large populations in various countries around the world.[1,2] A novel type of coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or 2019-nCoV), the causative agent of coronavirus disease 2019 (COVID-19), was first reported in Wuhan, Hubei Province, China, in December 2019.[2] During the COVID-19 pandemic, several viral lineages of various clinical and public health impact were identified, and divided into variants of interest (VOIs) and variants of concern (VOCs).[3–6]

The whole genome of SARS-CoV-2 contains 30 kb single-stranded ribonucleic acid (RNA) that encodes 29 different proteins. The most prominent structural proteins are spike (S), envelope (E), membrane (M), and nucleocapsid (N) ones. These proteins play a vital role in the pathogenesis of the virus, and each has distinct functions.[7]

The S protein is the major surface protein that binds to the host cell receptors and facilitates entrance into human cells; in approx. 86%, it is similar to the SARS-CoV protein. Moreover, it seems that mutations in the gene encoding the S protein cause differences in the pathogenic potential of SARS-CoV-2 as compared to SARS-CoV.[8]

The E protein is one of the small structural proteins involved in the viral life cycle and pathogenesis; it participates in virus assembly, budding and envelope formation.[9] The protein in SARS-CoV-2 is very similar to the SARS-CoV variant, with similarity estimated to be 94.74%.[10]

The M protein, as the most abundant glycoprotein, plays a critical role in virus size and shape maintenance, and assists in the assembly and budding stages of the virus. In addition, the M protein cooperates with the S protein to facilitate virus attachment and entry into host cells.[10] According to studies, the M protein of SARS-CoV-2 resembles the M protein of SARS-CoV in 90%, which means there is a remarkable similarity between the 2 types of virus.[11]

The N protein is a multi-purpose protein with several functions. It is involved in the viral life cycle, including virus core formation, assembly, budding, envelope formation, genomic messenger RNA (mRNA) replication, and genomic RNA synthesis. It is also vital for the cellular response, playing a role in chaperone activity, cell cycle regulation, cell stress responses, pathogenesis, and signal transduction.[12] In addition, the SARS-CoV-2 N protein has 90% similarity with the SARS-CoV N protein, which highlights its functional importance.[13]

Several vaccines were developed during the coronavirus pandemic and produced promising results. However, the high mutation rate of SARS-CoV-2 prompted the use of computational approaches to increase knowledge on the prevention and treatment strategies.[14,15] Although several SARS-CoV-2 epitopes have been reported in numerous vaccine development studies, the search for specific and unique epitopes has opened the door for future discoveries. Therefore, the present study aimed to identify and screen distinct epitopes of the M and N proteins of VOCs by using bioinformatics databases.

Material and methods

Sequences of membrane and nucleocapsid proteins, and the phylogenetic tree

Several sequences representing all the circulating VOCs of SARS-CoV-2, presented by the World Health Organization (WHO) (https://www.who.int/activities/tracking-SARS-CoV-2-variants), including GenBank accession numbers OX008586.1, OL790194.1, OW998408.1, OW996240.1, ON286809.1, ON286831.1, and OX014251.1, were acquired from the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov). The sequences were saved in the FASTA format, and a phylogenetic tree was designed using Molecular Evolutionary Genetics Analysis Version 11 (MEGA 11). The FASTA format of the SARS-CoV-2 wild-type (WT) (Wuhan-Hu-1) strain (accession number NC_045512.2) was included for comparison. The conserved regions of the proteins were identified and evaluated for further work.

Prediction of T-cell epitopes

In order to predict the cellular immunoreactivity of epitopes, several online servers, including NetMHC (https://services.healthtech.dtu.dk/service.php?NetMHC-4.0), Immune Epitope Database (IEDB) (https://www.iedb.org), NetCTL (https://services.healthtech.dtu.dk/service.php?NetCTL-1.2), MHC2Pred (http://crdd.osdd.net/raghava/mhc2pred), and SYFPEITHI (http://www.syfpeithi.de), were used. The epitopes with the highest scores for intensely stimulating the cellular immune system were selected for the study.

Validation of B-cell epitopes

The B-cell epitopes of the M and N proteins, containing at least 10 amino acids were predicted by IEDB and the artificial neural network-based B-cell epitope prediction server (ABCpred) (https://webs.iiitd.edu.in/raghava/abcpred), with threshold values of 0.5. Next, the selected epitopes were assessed based on their hydrophilicity, flexibility, polarity, surface area, and three-dimensional (3D) structures.

Evaluation of allergenicity and antigenicity

The allergenicity and antigenicity of the sequences were evaluated using AllerTOP (https://www.ddg-pharmfac.net/allertop) and VaxiJen (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), respectively.

Structure and docking analysis

The physicochemical and biochemical properties of the epitopes, including molecular weight, stability and hydrophobicity, were characterized using the ProtParam tool (https://web.expasy.org/protparam), and the 3D structures of peptides were drawn using the Molegro Virtual Docker software, v. 6.0.1 (Molegro ApS, Aarhus, Denmark). To analyze the molecular docking interaction with the highly-replicated epitopes, the tertiary structures of human leukocyte antigen (HLA) were gained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (https://www.rcsb.org). Then, the best ligand with minimum energy was assessed through Molegro Virtual Docker.

Results

The phylogenetic analysis and multiple sequence alignment of the complete SARS-CoV-2 genome revealed a close relationship between different variants of the virus (Supplementary material available on request from the corresponding author). Protein Basic Local Alignment Search Tool (BLAST) findings in NCBI also showed that the M and N proteins were highly conserved among the SARS-CoV-2 subtypes.

Stimulation of the cellular and humoral immune system

Of all the M epitopes identified in this study, LVIGFLFLT, WLLWPVTLA, LFLTWICLL, and FLYIIKLIF were capable of evoking cellular immunity, and seemed to induce a stronger host response. Also, they could correctly bind to the major histocompatibility complex class I (MHC-I) located at positions 22–30, 55–63, 27–35, and 45–53, respectively (Table 1). After immunogenicity and allergenicity evaluation, the final epitopes, including LVIGFLFLT and LFLTWICLL, were predicted as candidates for vaccine development.

The sequences KLDDKDPNFKDQ, RGPEQTQGNFGD and HIGTRNPANNAA, were identified as the frequented epitopes of the N protein that could interfere with cellular and humoral immune responses (Table 2). Based on the evaluation with the use of different servers, the best epitope of the N protein that could stimulate B-cells was KLDDKDPNFKDQ, located at position 338–349 of the sequence. Conversely, none of the M protein epitopes could elicit a robust humoral immune response (Table 3).

Table 1. List of the high-scored major histocompatibility complex class I (MHC-I) predicted epitopes of the membrane (M) protein

Epitopes	Alleles	Servers	AllerTOP results	VaxiJen results
LVIGFLFLT	HLA-B44:03, HLA-B44:02, HLA-B07:02, HLA-B08:01, HLA-A24:02, HLA-A01:01, HLA-B15:01, HLA-B35:01, HLA-B40:01, HLA-B53:01, HLA-A23:01, HLA-B51:01	NetMHC IEDB NetCTL MHC2Pred SYFPEITHI	probable non-allergen	probable antigen
WLLWPVTLA	HLA-B44:02, HLA-B44:03, HLA-B57:01, HLA-B58:01, HLA-A24:02, HLA-B53:01, HLA-A23:01, HLA-B35:01, HLA-B40:01, HLA-A68:01, HLA-A31:01	NetMHC IEDB NetCTL MHC2Pred SYFPEITHI	probable allergen	probable antigen
LFLTWICLL	HLA-A01:01, HLA-A68:01, HLA-B40:01, HLA-B44:03, HLA-B15:01, HLA-A11:01, HLA-A26:01, HLA-B44:02, HLA-A03:01, HLA-B35:01, HLA-B07:02, HLA-A68:02, HLA-B53:01	IEDB NetCTL MHC2Pred SYFPEITHI	probable non-allergen	probable antigen
FLYIIKLIF	HLA-A68:02, HLA-A30:01, HLA-A11:01, HLA-A68:01, HLA-B44:03, HLA-A31:01, HLA-B44:02, HLA-A33:01, HLA-B07:02, HLA-B40:01, HLA-A03:01, HLA-A02:06, HLA-A02:03, HLA-A01:01, HLA-B51:01	IEDB NetCTL MHC2Pred SYFPEITHI	probable non-allergen	probable antigen

IEDB – Immune Epitope Database.

Table 2. List of the high-scored major histocompatibility complex class I (MHC-I) predicted epitopes of the nucleoplastic (N) protein

Epitopes	Alleles	Servers	AllerTOP results	VaxiJen results
KLDDKDPNFKDQ	HLA-B4001, HLA-A0101, HLA-A0201, HLA-A0301, HLA-A2402, HLA-A2601, HLA-B2705, HLA-B3901, HLA-B5801, HLA-B1501	NetMHC	probable non-allergen	probable antigen
	HLA-A32:01, HLA-B08:01, HLA-A02:03, HLA-A02:01, HLA-A68:02, HLA-A26:01, HLA-A02:06, HLA-A33:01, HLA-A23:01, HLA-A30:01, HLA-B57:01, HLA-A30:02, HLA-A03:01, HLA-A24:02, HLA-B51:01, HLA-A02:01, HLA-B35:01	IEDB		
	HLA-A01, HLA-A02:01, HLA-A03, HLA-A11:01, HLA-A24:02, HLA-A26, HLA-A68:01, HLA-B07:02, HLA-B08, HLA-B13, HLA-B14:02, HLA-B15:01, HLA-B15:10, HLA-B15:16, HLA-B18, HLA-B27:05, HLA-B27:09, HLA-B35:01, HLA-B37, HLA-B38:01, HLA-B39:01, HLA-B39:02, HLA-B40:01, HLA-B41:01, HLA-B44:02, HLA-B45:01, HLA-B47:01, HLA-B49:01, HLA-B50:01, HLA-B51:01, HLA-B53:01, HLA-B57:01, HLA-B58:02	SYFPEITHI		
RGPEQTQGNFGD	HLA-B4001, HLA-A0101, HLA-A0201, HLA-A0301, HLA-A2402, HLA-A2601, HLA-B0702, HLA-B0801, HLA-B2705, HLA-B3901, HLA-B5801, HLA-B1501	NetMHC	probable non-allergen	probable antigen
	HLA-B57:01, HLA-B08:01, HLA-A30:02, HLA-A30:01, HLA-A01:01, HLA-B53:01, HLA-B40:01, HLA-B35:01, HLA-B15:01, HLA-B07:02, HLA-A68:02, HLA-A68:01, HLA-A33:01, HLA-A32:01, HLA-A31:01, HLA-A26:01, HLA-A24:02, HLA-A11:01, HLA-A03:01, HLA-A02:06, HLA-A02:03, HLA-A02:01	IEDB		
	HLA-A01, HLA-A02:01, HLA-A03, HLA-A11:01, HLA-A24:02, HLA-A26, HLA-A68:01, HLA-B07:02, HLA-B08, HLA-B13, HLA-B14:02, HLA-B15:01, HLA-B15:10, HLA-B15:16, HLA-B18, HLA-B27:05, HLA-B27:09, HLA-B35:01, HLA-B37, HLA-B38:01, HLA-B39:01, HLA-B39:02, HLA-B40:01, HLA-B41:01, HLA-B44:02, HLA-B45:01, HLA-B47:01, HLA-B49:01, HLA-B50:01, HLA-B51:01, HLA-B53:01, HLA-B57:01, HLA-B58:02	SYFPEITHI		
HIGTRNPANNAA	HLA-B40:01, HLA-B44:03	IEDB	probable allergen	probable antigen
	HLA-B40:01, HLA-B35:01, HLA-A24:02, HLA-B51:01, HLA-B07:02, HLA-A02:03, HLA-B15:01, HLA-A02:01, HLA-A33:01, HLA-B44:02, HLA-A68:02, HLA-A26:01, HLA-A02:06, HLA-A32:01	SYFPEITHI		

Table 3. Most frequent B-cell epitopes of the nucleocapsid (N) protein

Epitopes	Servers	AllerTOP results	VaxiJen results
KKDKKKKADETQ	ABCpred	probable non-allergen	probable antigen
HIGTRNPANNAA		probable allergen	probable antigen
PSDSTGSNQNGE		probable non-allergen	probable antigen
GTGPEAGLPYGA		probable allergen	probable antigen
QRRPQGLPNNTA		probable non-allergen	probable non-antigen
NSSRNSTPGSSR		probable non-allergen	probable antigen
ELIRQGTDYKHW		probable allergen	probable non-antigen
KLDDKDPNFKDQ		probable non-allergen	probable antigen
RIRGGDGKMKDL	IEDB	probable non-allergen	probable antigen
GTGPEAGLPYGANK		probable non-allergen	probable antigen
ALNTPKDHIGTRNPANN		probable allergen	probable antigen
AFGRRGPEQTQGNFG		probable allergen	probable antigen
EVTPSGTWL		probable non-allergen	probable non-antigen
KLDDKDPNFK		probable non-allergen	probable antigen

ABCpred – artificial neural network-based B-cell epitope prediction server.

Determination of structural conformations and molecular docking

According to the ProtParam server, 3 epitopes were defined as stable peptides, and the molecular weight, isoelectric point and hydrophobicity were 1,462.58 g/mol, 4.58 and 2.308 for KLDDKDPNFKDQ, 1,022.30 g/mol, 5.52 and 2.733 for LVIGFLFLT, and 1,121.45 g/mol, 5.52 and 2.600 for LFLTWICLL, respectively.

The 3D structures of the best peptides are shown in Fig. 1. The predicted results obtained from RCSB PDB showed that the epitopes could interact with HLA and induce strong immune responses. Moreover, the findings showed that there was an interaction between MHC-I/II (HLA-A0201:KLDDKDPNFKDQ, HLA-B51:LFLTWICLL and HLA-DRB1:LVIGFLFLT) and the suggested epitopes (Fig. 2).

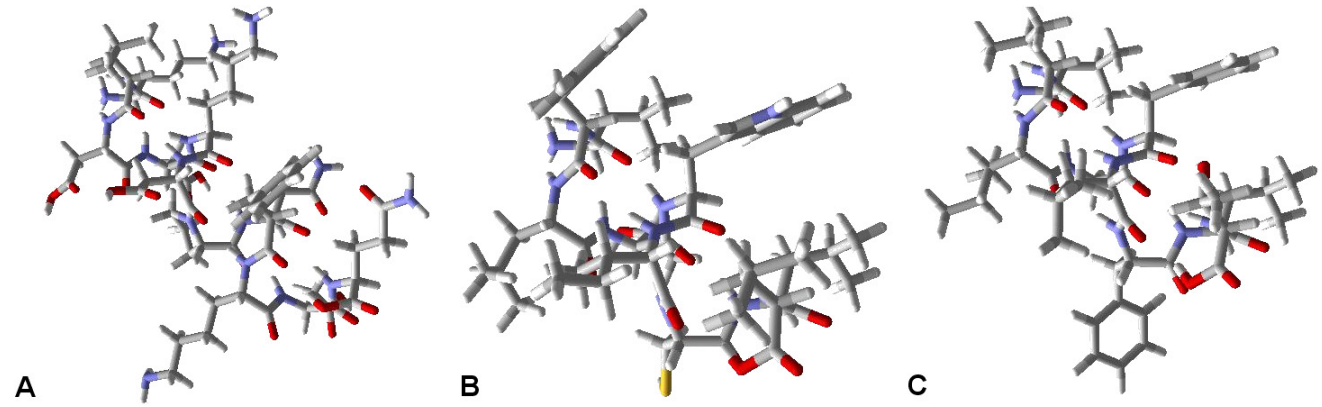

Fig. 1. Structures of the 3 epitopes, designed by means of the Molegro Virtual Docker software
A – KLDDKDPNFKDQ; B – LFLTWICLL; C – LVIGFLFLT.

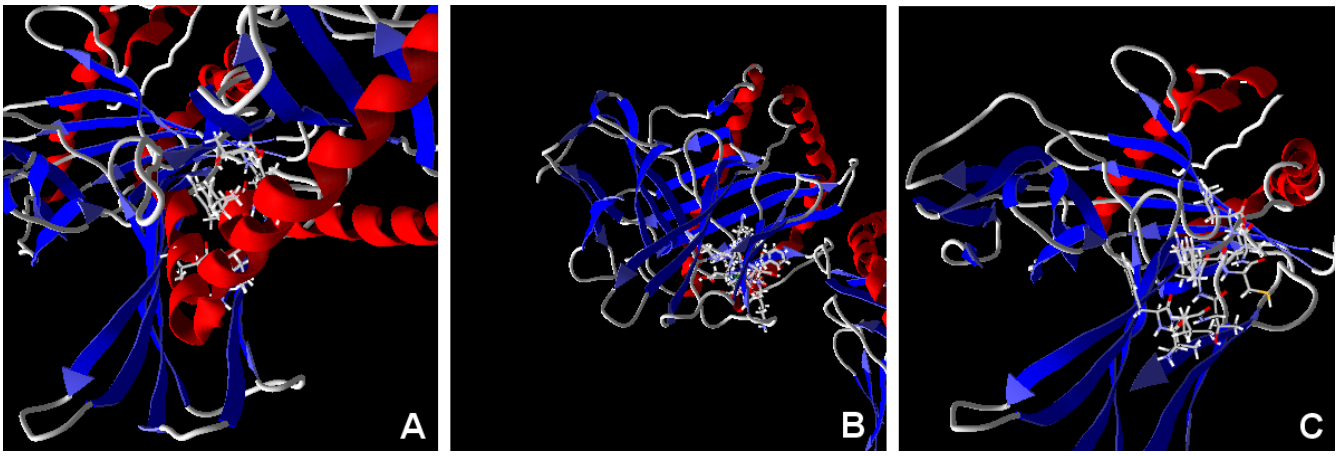

Fig. 2. Docking interaction and bond formation between the types of human leukocyte antigen (HLA) and the 3 epitopes, obtained using Molegro Virtual Docker
A – HLA-A0201 and KLDDKDPNFKDQ; B – HLA-B51 and LFLTWICLL; C – HLA-DRB1 and LVIGFLFLT.

Discussion

COVID-19 has been reported in many parts of the world as having a wide collection of clinical symptoms, ranging from fever and a dry cough to respiratory involvement, heart failure and kidney damage.[16–18] The increasing mortality, especially in patients with underlying diseases, such as diabetes, cancer, cardiovascular complications, and bacterial co-infections, as well as the spread of viral mutations, emphasizes the importance of disease prevention and treatment.[19–22]

Bioinformatics databases have proven to be useful sources of information on disease prevention, especially in the case of the diseases associated with life-threatening infections. Furthermore, bioinformatics methods have created a new avenue for designing effective vaccines at a low cost and with high efficiency. Indeed, the knowledge available in the bioinformatics field makes it possible to design effective vaccines against viruses and other infectious agents through using extensive information about the structural and immune features of microorganisms and humans.[23,24]

An ideal vaccine should be able to activate both the cellular and humoral arms of the immune system. Different platforms are used for vaccine development, and each of them presents several advantages and disadvantages. Typically, the subunit vaccines using recombinant peptides and proteins are among the most effective, inexpensive and safe vaccines that can be designed. Moreover, this type of vaccine provides effective immunogenicity by evoking host immune responses.[25,26]

According to studies on the prevention of COVID-19, there are more than 60 vaccine candidates for SARS-CoV-2, most of which are aimed at inducing the release of neutralizing antibodies against the S protein.[27] Unfortunately, several reports have shown that the S protein is an antigen that can mutate rapidly. For example, the most common type of coronavirus, omicron, has more than 30 S protein mutations.[28–31] Furthermore, a number of reports have confirmed that the M and N proteins are good targets for stimulating the antibody-producing B-cell and T-cell responses.[32,33] Indeed, studies published by Enayatkhani et al.,[33] Rahman et al.[34] and Quayum et al.[35] emphasized the importance of the M and N proteins in the viral structure, confirming their potential role as suitable candidates for predicting multi-epitope vaccines. However, the current work focused on the M and N protein epitopes to predict a novel subunit vaccine.

In the present study, all epitopes were evaluated based on different immune responses, and the findings indicate that the M protein may be a useful target for eliciting a cellular immune response, while the N peptides could elicit a strong humoral immune response. Also, the results demonstrate that, among many epitopes, 2 highly antigenic M proteins, LVIGFLFLT and LFLTWICLL, and the KLDDKDPNFKDQ N protein could be used to construct an epitope-based vaccine. The LVIGFLFLT and LFLTWICLL M proteins were the best options among T-cell epitopes, while KLDDKDPNFKDQ was identified as a powerful B-cell epitope.

A number of publications reported on the LVIGFLFLT and LFLTWICLL M epitopes, including works by Behmard et al.[36] and Naveed et al.[37] In addition, Heffron et al. introduced KLDDKDPNFKDQ as part of the AIKLDDKDPNFKDQVI and KLDDKDPNFKDQVILLNKH peptides in a study on antibodies against the SARS-CoV-2 N protein.[38] In comparison with those studies, the findings of this study were more specific. Indeed, this work assessed specific epitopes to determine their distinct sites in the protein sequences, and introduced unique epitopes for vaccine and antibody research.

The present study included the SARS-CoV-2 WT (Wuhan-Hu-1) strain sequence and compared the results with other VOCs, especially lineage B.1.1.529, with respect to the worldwide omicron epidemic. All the suggested epitopes were matched with the new variants of COVID-19.

Conclusions

COVID-19 continues to be an alarming global disease, as observed in the reports of new cases and deaths, which increases the importance of developing a more effective vaccine. The immunoinformatics results obtained for the M and N proteins identified 3 top epitopes, including LVIGFLFLT, LFLTWICLL and KLDDKDPNFKDQ, that could effectively stimulate T-cells and B-cells with the lowest binding energy. Therefore, additional in vitro and in vivo studies are recommended to confirm this theoretical information. Moreover, bioinformatics tools are suggested to be used against future epidemics to design new vaccines for other infectious diseases, and researchers should pay more attention to this issue.

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

Strain gauge analysis and fracture resistance of implant-supported PEKK hybrid abutments restored with two crown materials: An in vitro study

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Abstract

Background. Polyetherketoneketone (PEKK) was recently introduced as an alternative to titanium and ceramic implant abutments due to its apparent ability to dissipate excessive strain around dental implants. However, the biomechanical behaviors of implant abutment crown systems may change depending on the crown and abutment material combinations used.

Objectives. This study aimed to assess how the crown material affects strain generation and fracture resistance of PEKK hybrid abutment crowns.

Material and methods. Sixteen dummy implants (Ø 3.7 × 11 mm), simulating maxillary first premolars, were restored with 16 milled PEKK hybrid abutments and randomly categorized into two groups according to the crown material ($n = 8$): Group C, milled composite crowns cemented on PEKK hybrid abutments; and Group Z, ultra-translucent zirconia crowns cemented on PEKK hybrid abutments. Before thermocycling, a cyanoacrylate-base adhesive was used to position two strain gauges on buccal and lingual crestal bone surfaces, and a vertical load (100 N) was applied to the central fossa to record the strain generated. Then, all samples were thermocycled between 5°C and 55°C before being loaded to fracture on a universal testing machine. Modes of failure were observed under an optical microscope, and representative samples were examined using a scanning electron microscope. Independent t-tests were used for intergroup comparisons. The significance level was set at ($p < 0.05$) for all tests..

Results. The results showed a significant difference between both groups. The zirconia group recorded significantly higher strain and fracture resistance values than the composite group ($p < 0.001$). There was a positive correlation between the strain developed in peri-implant crestal bone and fracture resistance of the abutment crown complex.

Conclusions. Strains developed in both groups were within the acceptable clinical range. The crown material substantially impacted the strain and fracture of the PEKK hybrid abutment crown system.

Keywords: dental implants, zirconia, composite resins, implant abutments, polyetherketoneketone

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Introduction

The high success rate of dental implants depends on good osteointegration and the overlying superstructure's performance.[1] However, dental implants may encounter biomechanical complications as they lack the dampening behavior of periodontal ligaments in natural teeth; thus, implants and natural teeth respond differently to typical masticatory forces.[2,3] Therefore, loading on dental implants should be controlled via the correct choice of implant, abutment, crown materials, and designs.[3]

Titanium abutments were the most commonly used implant abutments due to their superior biomechanical properties. However, their greyish color could be transmitted through the thin biotype gingiva and affect the final shade of ceramic restorations.[4,5] Therefore, dentists shifted to using more esthetic abutments and developed ceramic abutments. The first all-ceramic abutments used were zirconia due to their favorable esthetic and mechanical properties.[6] However, they encountered a critical problem that involved fracturing of the apical part during screwing.[7] Due to these problems, the concept of hybrid abutments was proposed.

Hybrid abutments consist of a non-metal part customized over a prefabricated titanium base, which provides a better esthetic restoration, and a titanium connection within the implant prevents fracture at the implant-abutment interface.[8,9] Modification of the non-metal part allows for better customization of the emergence profile and treatment of cases that require angulation correction. The presence of a cement layer between the titanium base and ceramic part acts as a weak link that decreases the possibility of screw looseness.[10] These advantages demonstrate that hybrid abutments could be effective in implant rehabilitation cases.

Hybrid abutment restorations can be screw-retained, cement-retained, or screwmentable designs. Screw-retained restorations are easily retrievable for the repair of fractures and have insignificant biological complications as no excess cement remains in the sulcus.[11–13] However, they carry a risk of screw loosening or ceramic fracture.[14,15] Cement-retained restorations can compensate for implant position discrepancies and have improved esthetics and better control of occlusion. The cement layer acts as a shock absorber that uniformly transfers loads to the implant prostheses bone complex.[12,13] Nevertheless, there is a risk of peri-implantitis due to excess cement not being adequately removed from the soft tissue.[15,16] The screwmentable design combines the benefits of both designs (screw-retained and cement-retained) by allowing intraoral adjustments of the crown and contact area during restoration delivery without multiple removal and replacement of the screw, and the crown can be extraorally cemented on the abutment, allowing excess cement to be easily removed.[13,17,18]

Different materials are available for use in hybrid abutments. Conventional ceramics, such as oxides (alumina, zirconia) and glass (lithium disilicate), can produce highly esthetic hybrid abutments; however, they have a high incidence of failure when used over implants, possibly caused by their rigidity.[19] While searching for a restorative material with a lower elastic modulus, high-performance polymers called polyaryletherketone (PEKK) have been developed.[5,20]

Compared to titanium, high-performance polymers showed a decreased marginal bone loss and soft tissue recession during the initial healing phase by reducing the occlusal loads reaching the bone.[21,22] Moreover, a previous study revealed that unpolished polymers had a lower surface roughness than zirconia abutments, which will provide less biofilm accumulation and better soft tissue attachment; however, they found that the flexibility of these materials may cause a higher strain in the implant and peri-implant bone.[23]

PEKK is a high-performance polymer claimed to have good shock absorption properties and high compressive strength due to the additional ketone group in its structure.[24] Nevertheless, PEKK has a monochromatic opaque appearance and is usually combined with an overlying esthetic crown. The manufacturer recommends restoring PEKK abutments with either composite or ceramic crowns.[25] Composite resin has a low elastic modulus that could be beneficial over implant restorations. Milled composite crowns showed no polymerization shrinkage and enhanced mechanical and wear properties over direct composite layering.[26,27] On the other hand, some authors claim that using a rigid crown material like zirconia would decrease the forces transmitted to the abutment and bone.[28]

Several tools were used in the literature to record the strain around dental implants, such as finite element analysis,[29,30] photoelasticity,[31] and strain gauges.[32,33] Strain gauges are electric resistors that alter the resistance created in their current under slight deformation. They can be used to assess strain developed in prostheses, implants, and teeth in vivo and in vitro.[32] Moreover, using a strain gauge to evaluate the strains induced in the implants is clinically reliabile.[34]

Due to the multilayer nature of the implant superstructure, the biomechanics of the implant system can vary depending on the crown and abutment material combinations, so the current study aimed to investigate the peri-implant crestal bone strain and fracture resistance of composite and zirconia crown materials when combined with PEKK hybrid abutments and fabricated as screwmentable restorations.

The null hypothesis of the present study stated that neither peri-implant marginal bone strain nor fracture resistance of the PEKK hybrid abutment crown complex would be affected by the crown material.

Material and methods

A power analysis was designed based on the results of a previous study.[35] By adopting an alpha (α) level of 0.05 (5%), a beta (β) level of 0.2 (20%) (i.e., power = 80%), and

effect size (d) of (1.65), the minimum required sample size (n) was seven per group. The sample size calculation was performed using G*Power version 3.1.9.4.

Sixteen dummy implants, Ø 3.7 x 11 mm (JDentalCare s.r.l, Modena, Italy) resembling the average implant dimensions in the premolar region,[36,37] were placed perpendicularly in self-cured resin (Technovit 4000, Heraeus Kulzer, Hanau, Germany), with an elastic modulus (12 GPa) that approximates that of trabecular bone (18 GPa). A paralleling device (Dremel® Moto-Tool Model 395, WI, USA) was used to maintain the implant in place until the resin was completely set.

A titanium base (JDentalCare s.r.l, Italy) with platform switching, 0.5 mm shoulder finish line, and 3 mm height was hand screwed to the dummy implant. The screw access channel was sealed with wax, and the titanium base was then sprayed with light-reflecting powder (Occlutec spray, Renfert, Hilzingen, Germany) to facilitate the scanning procedure. Scanning employed a desktop scanner (Medit T710, Medit Corp, Seoul, Korea). Computer-aided design (CAD) software (Exocad GmbH, Version 3.0, Darmstadt, Germany) was used to design the abutments and crowns. The abutment dimensions were adjusted following Taha et al.[38] The crowns were designed with a screw channel within the occlusal surface and adjusted according to the average dimensions of a maxillary first premolar, with a crown height of 11.5 mm and buccolingual width of 6.5 mm.[39] Then STL files of the design were sent from the CAD software to computer-aided manufacturing (CAM) software. A five-axis milling machine (Glidewell Dental Labs, VHF Camfacture AG, Ammerbuch, Germany) was used to mill the abutments and the crowns.

The abutments were divided into two groups (n=8) according to the crown material: milled composite (Group C) and zirconia (Group Z). PEKK blanks (Pekkton® ivory, Cendres+Métaux, Milano, Italia) were used to fabricate the PEKK abutments for both groups. Composite blocks (Brilliant Crios, Coltène AG; Altstätten, Switzerland) were used to fabricate the composite crowns in Group C. Zirconia blanks (UTML, Kuraray Noritake, Japan) were used to fabricate zirconia crowns in Group Z.

After milling, the PEKK abutments were finished and polished using a specific kit (BioHPP polishing kit, Bredent, Germany), while zirconia crowns were sintered and glazed using a zirconia sintering furnace (inFire HTC speed, Sirona Dentsply, Bensheim, Germany) at 1550°C for two hours. For the composite crowns, finishing and polishing were done using a two-stage polishing system (Diatech polishing kit, Coltène AG; Altstätten, Switzerland). The titanium bases, milled abutments, and crowns were cleaned in an ultrasonic bath, degreased with ethanol, and then treated according to the manufacturer's recommendations, as shown in Table 1.

Table 1. Surface treatment of the materials used in the study

Component	Surface treatment
Titanium bases	sandblasting with 50 µm aluminum oxide at 2.5 bar pressure and 10 mm distance, then priming with Monobond Plus (Ivoclar Vivadent, Schaan, Liechtenstein) for 60 s
PEKK abutments	sandblasting with 110 µm aluminum oxide at 3 bar pressure, then moistening with Visio.Link primer (Bredent, Senden, Germany) for 10 s
Zirconia crowns in group Z Milled composite crowns in group C	sandblasting with 50 µm aluminum oxide at 2 bar pressure and a distance of 10 mm for 30 s, followed by the application of a universal primer (Monobond Plus) for 60 s

The screw channel was sealed with Teflon tape, and Multilink Hybrid Abutment self-cured resin cement (Ivoclar Vivadent; Schaan, Liechtenstein) was applied to the titanium bases. The pretreated abutments were cemented to their corresponding titanium bases. The abutments were first seated with finger pressure and then left to set under a 5 Kg load.

The intaglio surfaces of the crowns were sandblasted and treated with universal primer (Monobond Plus primer, Ivoclar Vivadent; Schaan, Liechtenstein), as mentioned in Table 1. An adhesive resin cement (Breeze™, Pentron Clinical, CA, USA) was used to cement the crowns to the abutments. A static load was applied over the cemented crowns for five minutes. The prostheses were tightened to the implants using a screwdriver and torque wrench under 30 Ncm to avoid preload screw loosening then they were retightened after ten minutes. Teflon tape was placed to seal the screw channels, and then a light-cured composite (Filtek™ Z250) was applied.

Two strain gauges (Kyowa Kirin, Koyowa, Japan) (Ø1 mm) were positioned on the buccal and lingual crestal surfaces of the bone analog with a delicate layer of cyanoacrylate-base adhesive cement (Super glue, China).

Before thermocycling, a universal testing machine (LLOYD Universal Testing Machine, UK) applied a vertical load (100 N) onto the central fossa to record the strain generated in the peri-implant crestal bone. Tin foil was fixed between the loading piston and the specimen to ensure equal strain distribution.

All samples were artificially aged in a thermocycler (Thermocycler, SD Mechatronik, Feldkirchen-Westerham, Germany) to a total of 5000 thermal cycles between 5°C and 55°C with a dwell time of 30 seconds and a transfer time of five seconds, which is equivalent to six months in the oral environment.[37]

The specimens were loaded with vertical loads (1 mm/min) onto the central fossa until fracture.[40] The fractured specimens were examined with an optical microscope (Dino-Lite Electronic Corp., Taiwan) at 35× magnification to analyze the mode of failure. Representative specimens were sputtered with gold for five minutes and observed with a scanning electron microscope (SEM) (Neoscope (JCM-6000 Plus), JEOL, Japan) at 17× magnification and then at 100× magnification to detect the cause of fracture.

Statistical analysis

Numerical data were explored for normality by assessing the data distribution using a Shapiro-Wilk test. The data were normally distributed and were represented as mean and standard deviation (*M* ±*SD*). Independent *t* tests were used for intergroup comparisons. Pearson's correlation coefficient was used to study the correlation between fracture resistance and strain. The significance level was set at $p < 0.05$ for all tests. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows (R Core Team, 2022).

Results

The results revealed that the zirconia group had significantly higher strain and fracture resistance values than the composite group ($p < 0.001$) (Table 2).

There was a strong positive correlation between strain falling on the implant surrounding marginal bone and the fracture resistance of the implant superstructure. The relationship was statistically significant (r = 0.970; $p < 0.001$).

Failure modes were classified as Class I: a fracture in the crown only, Class II: a fracture in the abutment only, Class III: a fracture in the crown and abutment, and Class IV: screw fracture and implant deformation.[41] In Group C, Class I failure was observed in all specimens without fractures or deformations within the abutments (Fig. 1A). For Group Z, failures were more catastrophic and were within the abutments and crowns (Class III). The failure exhibited bending and deformation of the abutment and vertical fracture of the abutment and crown (Fig. 2A).

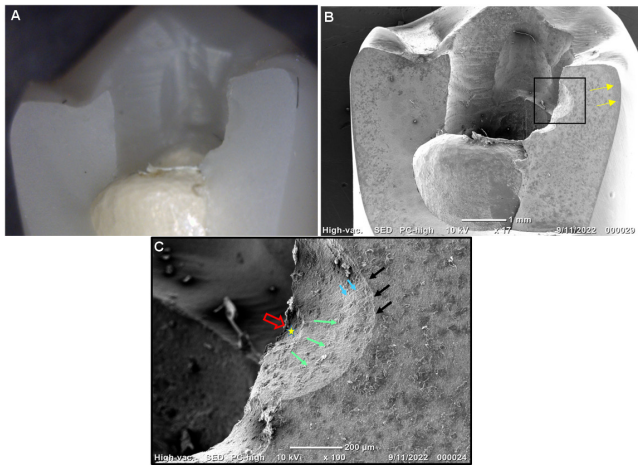

Fig. 1. Mapping of fracture in group C

A – optical image of a fractured sample in group C shows fracture within the composite crown only; B – SEM image 17× shows the signs of fracture along the screw channel and the compression curl is represented by the yellow arrows. The black rectangle is magnified to image C; C – in the magnified black rectangle 100×, several cracks along the screw channel are noticed; the crack beginning (red arrow), smooth zone of fracture (yellow star), hackle lines (green arrows), twist hackles (blue arrows) and an arrest line (black arrows) are observed.

Table 2. Strain [μm/μm] and fracture resistance [N] for both groups

Measurement	Composite crown (group C)	Zirconia crown (group Z)	*p*-value
Strain	307.60 ±35.16	1,242.81 ±109.56	<0.001*
Fracture resistance	351.12 ±46.56	733.28 ±60.22	<0.001*

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

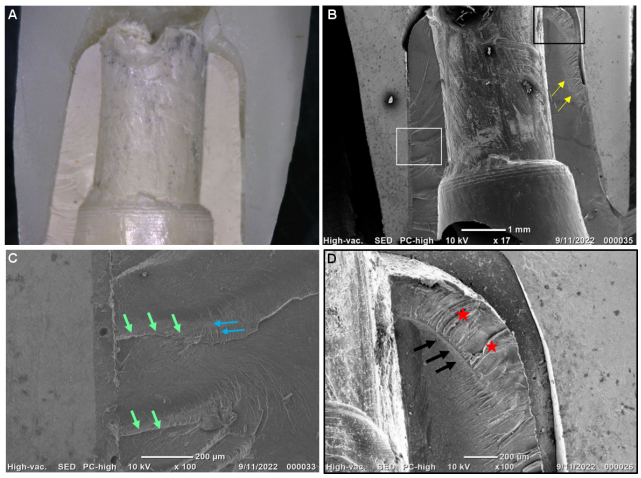

Fig. 2. Mapping of the fracture in group Z

A – optical image of a fractured sample in group Z shows fracture of both the zirconia crown and PEKK abutment; B – SEM image 17× shows multiple cracks within PEKK abutment and the compression curl is represented by the yellow arrows. The white rectangle is magnified to image C and the black rectangle is magnified to image D; C – magnified white rectangle 100× shows several main cracks (green arrows) within the PEKK abutment and twist hackles (blue arrows) radiating in an upward direction, suggesting that the fracture originated from the abutment and extended to the overlying crown; D – magnified black rectangle 100× shows signs of bending and plastic deformation (red stars) within the PEKK abutment with the tension side towards the zirconia crown and the compression side inwards. An arrest line is observed on the compression side (black arrows).

Fractographic analysis for Group C under SEM showed that the fracture originated from the screw channel and propagated as small cracks within the crowns without any cracks or plastic deformation in the PEKK abutments (Fig. 1B,C). In Group Z, multiple cracks and signs of bending were observed within the PEKK abutments (Fig. 2B,C,D).

Discussion

There has been much debate on whether rigid or soft implant crown material will transfer lower strains to the implant-bone surrounding,[26–28,38] so this study was based on biomechanical evaluation of peri-implant cervical bone strain and fracture resistance of implant-supported prostheses, fabricated as screwmentable restorations, using two crown materials with substantial differences in their mechanical properties.

The null hypothesis of the study was rejected since strain analysis results revealed that Group C had significantly lower microstrain values than Group Z. The statistical difference could be attributed to the difference in elastic mod-

ulus between the crown materials (about 10 GPa for composite and 200 GPa for zirconia). The high elastic modulus of zirconia (200 GPa) lowered its damping behavior, which transmitted high forces to the substructure and bone. This agrees with Datte et al.,[42] who stated that increasing crown material stiffness increases the generated strain in the implant surrounding bone while decreasing the developed strain in the restoration itself.

Despite the strain analysis results, the two groups had a similar clinical effect on the peri-implant bone strain, as all values were within the clinically accepted range for microstrain (50-3000 μm) that enhances bone formation and prevents its overload or disuse.[43] The resin-based crown material acts as a shock absorber for the occlusal loads, making the strains developed in the bone more bearable.[38] For the zirconia group, combining a ceramic crown with a high elastic modulus in contact with the applied load and a material with a lower elastic modulus below the crown mimics the enamel and dentine behavior in natural teeth.[44] As such, even with the high elastic modulus of zirconia crowns, the zirconia-PEKK combination generated strains within the generally accepted limits. Another explanation suggests that the intermediate resilient cement layer improves the dampening behavior of the rigid crown materials, leading to better dissipation of the occlusal forces.[38]

Fracture resistance results support the first finding in the present study, as the material with high elastic and flexure modulus could not be easily deformed under loads. However, it would transfer high strains to the underlying structure, which was supported by the SEM findings showing cracks in the underlying PEKK abutments in Group Z (Fig. 2).

On the other hand, the material with low flexure and elastic modulus (composite) deformed under the applied load, preventing it from reaching the underlying abutment and implant-bone surroundings, so no deformations were observed in the composite group (Fig. 1). This was in line with Bijjargi and Chowdhary,[45] who concluded that using crown material with a low elastic modulus reduces the strains transferred to the underlying structure by absorbing more energy from the applied load while transmitting less energy to the implant, abutment, and bone.

Another explanation for the lower fracture values in Group C was the presence of the screw access channel within the crown. The screw access channel disrupted the structural integrity of the crown material, affecting its fracture resistance, particularly when the crown was formed of a material with a weak microstructure like composite. This agrees with a previous finding in a study by Preis et al.,[35] who found that screw channels might affect the continuity of resin-based occlusal material and lead to fracture. Our interpretation was also confirmed by the SEM findings, as the fracture origin for Group C was found to be along the screw channel, with needle-like cracks, called hackle lines, extended downward, which indicated the direction of crack propagation (Fig. 1). Therefore, the fracture originated and propagated from the screw channel.

On the other hand, in Group Z, stiff zirconia crowns (200 GPa) transferred higher strains to PEKK abutments with an elastic modulus of 5 GPa, which led to bending and deformation of the abutments and so concentrated high tensile strains on the overlaying brittle zirconia crowns and caused crown fracture. This was confirmed by the SEM findings (Fig. 2), which revealed signs of plastic deformation and bending in the PEKK abutment with the tension surface towards the zirconia superstructure and the compression surface inside.

The plastic deformations and bending occurred despite the PEKK microstructure containing an extra ketone group. However, this might be due to the fact that PEKK does not contain reinforcing elements to increase its stiffness (modulus of elasticity) and resistance to bending. The titanium oxides included in PEKK only increase their wear resistance,[46] which explains why when zirconia crowns transferred high strains to the PEKK abutments, they showed multiple signs of bending and plastic deformation. This finding was consistent with a previous study designed by Türksayar and Atsü,[47] showing that unmodified PEKK implant abutments had fracture load values less than those made of modified PEKK; however, they only tested one crown material.

Ghodsi et al.,[48] classified the fracture modes into favorable and unfavorable failures. A favorable failure occurs within the crown material while the abutment remains intact. An unfavorable failure is a fracture within the abutment that necessitates restoration or replacement. In the present study, despite Group Z having higher fracture values than the indirect composite, it showed more unfavorable catastrophic fractures than the composite group. However, having a customized abutment made from a material with a low elastic modulus protected the implant and titanium bases from being deformed or fractured in both groups. The indirect composite fracture was in the form of cracks or chipping of the composite crown without any fracture in the abutment, resulting in a more favorable mode of failure with an easy repair advantage. Nevertheless, composite resins are more prone to material wear, color instability, and loss of surface gloss than zirconia.[19,49]

Statistical analysis of the results clarified a strong positive correlation between the fracture resistance of the implant crown material and the microstrain produced in the implant surrounding the marginal bone. This might be due to the fact that both the fracture resistance of a material and its damping behavior are strongly related to Young's modulus.

Concerning the above, it could be hypothesized that stiff crown materials have high fracture resistance, increasing their ability to withstand high occlusal forces. However, these high loads could be transmitted from the crown to the underlying PEKK abutment and cervical peri-implant bone, although, in the current study, the loads were within the acceptable physiological range. Moreover, using a hybrid abutment with a low elastic modulus (PEKK) under rigid crown material could be beneficial as it would protect the underlying titanium bases, implant fixture, and peri-implant bone from being overloaded.

A limitation of the current study was that the tested specimens did not undergo cyclic loading, so further studies are required after mechanical aging.

Conclusions

Based on the findings of the present study, strains generated in both zirconia-PEKK hybrid abutment crowns and milled composite-PEKK hybrid abutment crowns were within the acceptable clinical range. Also, zirconia-PEKK hybrid abutment crowns can be used safely in the upper premolar region; however, milled composite-PEKK hybrid abutment crowns may cautiously serve in this region. Crown material had a statistically significant impact on the peri-implant marginal bone strain and fracture resistance of the PEKK hybrid abutment crown complex. Furthermore, there was a strong correlation between the fracture strength of crown material and the amount of force transferred to the peri-implant marginal bone.

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 tensile modulus of three 3D-printable materials used in dentistry

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Abstract

Background. Three-dimensional (3D) printing technology has brought much innovation to medicine and has been successfully adopted in many areas of dentistry. Although 3D printing techniques are being increasingly used, their advantages and disadvantages still need to be investigated, particularly with regard to the materials used in dentistry. Dental materials should be biocompatible and non-cytotoxic, and have sufficient mechanical integrity in the oral environment in which they are intended for use.

Objectives. The present work aimed to identify and compare the mechanical properties of three 3D-printable resins. The materials included IBT Resin, BioMed Amber Resin and Dental LT Clear Resin. The Formlabs Form 2 printer was used.

Material and methods. A tensile strength test was performed on 10 specimens of each resin. Tensile modulus was measured on 2-millimeter-thick dumbbell-shaped specimens, 75 mm in length and 10 mm in width. The 10 specimens of each resin were mounted between the grips of a universal testing machine (Z10-X700).

Results. The results showed that BioMed Amber specimens cracked easily, yet no deformation was observed. The amount of force used to test the tensility of the specimens was the lowest for IBT Resin, while it was the highest for Dental LT Clear Resin.

Conclusions. IBT Resin was the weakest material, whereas Dental Clear LT Resin was the strongest.

Keywords: dentistry, 3D printing, resins, tensile modulus

Introduction

Three-dimensional (3D) printing is becoming more and more popular in today's world, including the fields of medicine and dentistry. In dentistry, it has been applied for preparing prosthetic devices and conservative restorations. It also finds a use in other branches of dentistry, e.g., for manufacturing surgical guides, anatomical models and custom-made parts. The high print precision allows the development of perfectly fitting elements that are needed to restore the tissues. Although printable materials and traditional ones have similar properties, the former help to reduce chair time. The greatest disadvantage of this technology is its high total cost, as additional scanners and printers are needed.[1]

Although the 3D printing technology is developing quickly, some limitations are present, and the need for improvement is high. The search for an ideal restoration material is based on comparing various materials, especially in terms of their biocompatibility and resistance to general conditions. Esthetics is also taken into account.[2–4]

More advanced techniques, such as surgical planning, involve cone-beam computed tomography (CBCT) scanning. The most common procedure aided by 3D printing and CBCT is the preparation of surgical guides for the insertion of implants and orthodontic mini-implants. These guides help to find a precise implantation route, thus reducing the risk of tooth root damage, especially when there is little space between the roots.[1,5,6] The scan precision depends on the skills of the operator. With respect to the place of the tooth in the arch, more precise guides can be obtained at the buccal segments of the arch, as for premolars and molars, scanning is highly accurate. In the case of incisors, the scanner may fail to recognize their surfaces, as they are less characteristic as compared to other teeth.[7,8] Nonetheless, the advantages of using surgical guides outbalance the risk of potential complications and failure.

Apart from their use as an aid in surgical procedures, dental scans are performed to prepare casts for prosthodontic and conservative restorations.[9,10] The copings and frameworks for tooth restorations which are prepared with the use of highly precise special software perfectly fit the anatomical space.[11] Such techniques are termed computer-aided design/computer-aided manufacturing (CAD/CAM).[12] Although 3D printing is very common in surgery, prosthodontics and conservative dentistry, its applications are far more extensive. In orthodontics, 3D printing is most commonly used for preparing aligners and orthodontic trays for indirect bracket bonding. The techniques can also be used for manufacturing other customized items, such as occlusal splints and Michigan splints.[13] Other types of appliances can also be printed, including very precise items, like distalizers, or individual appliances used in more severe cases, such as palatal plates or obturators for cleft lip and/or palate patients.[14,15]

3D-printable materials appear to be appropriate for use. However, since they are exposed to oral cavity conditions for a long period of time, many factors, including food, beverages, as well as compressive and tensile forces, influence their properties, which should be considered before treatment planning. Indeed, external factors may lead to unfavorable changes in the properties of the materials, such as color fading or darkening, decreased hardness, and hence decreased resistance to breaking.[4,16–18] This issue is of utmost importance when considering dental restorations, but also when the long-term wear of splints is taken into account. As the reaction of the material to external conditions may change its properties, it may also affect its usage.[17–19] Moreover, it turns out that one change may evoke another, e.g., the color change correlates with changes in the microhardness, roughness and texture of the material.[18,20]

Among the producers of 3D-printable materials, Formlabs has found its place as a manufacturer of biocompatible resins. Their materials include IBT Resin, BioMed Amber Resin and Dental LT Clear Resin, which are novel and commonly used. According to the manufacturer, the transparent resins BioMed Amber and Dental LT Clear are strong, rigid and biocompatible. BioMed Amber has a yellowish glow, while Dental LT Clear is highly esthetic. BioMed Amber consists of several chemical compounds, including 7,7,9(or 7,9,9)-trimethyl-4,13-dioxo-3,14-dioxa-5,12-diazahexadecane-1,16-diyl bismethacrylate, 2-hydroxyethyl methacrylate and phenyl bis(2,4,6-trimethylbenzoyl)-phosphine oxide. The chemical composition of Dental LT Clear is more complex, and comprises 7,7,9(or 7,9,9)-trimethyl-4,13-dioxo-3,14-dioxa-5,12-diazahexadecane-1,16-diyl bismethacrylate, 2-hydroxyethyl methacrylate (Note D), reaction mass of bis(1,2,2,6,6-pentamethyl-4-piperidyl) sebacate, methyl 1,2,2,6,6-pentamethyl-4-piperidyl sebacate, diphenyl(2,4,6- trimethylbenzoyl)phosphine oxide, acrylic acid, monoester with propane-1,2-diol, ethylene dimethacrylate, 2-hydroxyethyl acrylate, mequinol, 4-methoxyphenol, and hydroquinone monomethyl ether. The properties of the third resin (IBT) differ – it is elastic and flexible, although transparency and translucency remain. The IBT has optimized tear strength.[17,21]

The present study aimed to compare the behavior of 3 materials designed for medical use by subjecting them to pressure and tension tests to increase knowledge of 3D-printable materials. Such comparison of biomechanical properties is not a commonly discussed issue. The current study is part of a series of papers prepared previously by the authors.[17,21]

Material and methods

The study compared 3 selected materials 3D-printed with the use of the Formlabs Form 2 printer (Formlabs, Sommerville, USA), which is dedicated for medical purposes. The examined materials were IBT Resin (Formlabs), BioMed Amber Resin (Formlabs) and Dental Clear LT Resin (Vertex-Dental, Soesterberg, the Netherlands). All of them are designed for medical uses, mainly in dentistry, though the literature on them is poor as compared to other dental materials. Therefore, the authors of this study decided to plan and present novel research, comparing the 3 resins recently introduced for use in dentistry.

Table 1 summarizes the manufacturers' recommendations regarding the applications of the selected dental resins.

The Formlabs Form 2 printer is self-adjustable, and all printing parameters were built into a chip in the resin container. All prints were performed using a Class 1 405-nm violet laser (250 mW) at a temperature of 35°C. The printing layer thickness was 100 µ for all resins. Printing was conducted according to the manufacturers' specifications and printer settings.

A tensile strength test was conducted for the printed specimens, with tensile modulus measured on 2-millimeter-thick dumbbell-shaped samples of 75 mm in length and 10 mm in width (type 1BA), following the ISO 527-2:2012 standard[22] (Fig. 1). The samples were air-incubated at 23°C and 50% relative humidity (RH) for 24 h after printing. Then, their height, width and length were measured at 5 points with the use of a Magnusson Vernier digital caliper (Limit, Wroclaw, Poland). The mean values were calculated. Subsequently, the specimens were mounted between the grips of a universal testing machine (Z10-X700; AML Instruments, Lincoln, UK). The test was performed at a speed of 5 mm/min, and the force was measured until each specimen broke. Only the section between the widened parts of the sample was tested, and specimens that broke outside of this area were discarded.

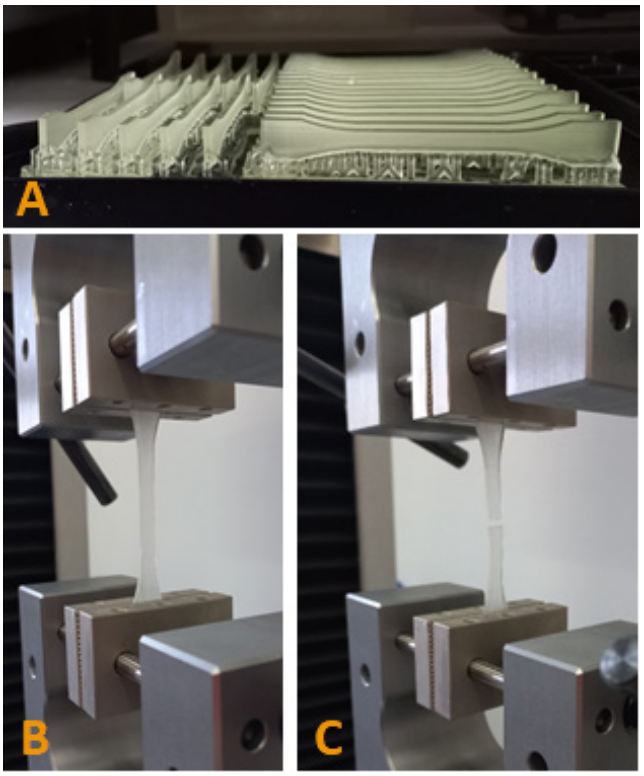

Fig. 1. Tensile strength test

A – set of specimens after printing; B – finished specimen mounted between the grips before the application of a tensile force; C – specimen broken by a tensile force.

Table 1. Brief description of the applications of the selected 3D-printable resins, recommended by the manufacturers

Resin	Applications
Dental LT Clear Resin	long-term biocompatible resin ideal for hard splints, occlusal guards, and other direct-printed long-term orthodontic appliances
BioMed Amber Resin	biocompatible applications requiring short-term skin or mucosal membrane contact; suitable for strong, rigid parts, such as functioning threads, end-use medical devices, cut + drill guides, implant-sizing models, and specimen collection kits
IBT Resin	ideal for manufacturing indirect bonding trays for a cost-effective, rapid dental bracket placement process for high-quality orthodontics

Tensile stress and nominal strain were calculated according to the following formulas (Equations 1,2):

$$\sigma = \frac{F}{A} \quad (1)$$

where:
σ – tensile stress [MPa];
F – force [N]; and
A – initial cross-sectional area [mm^2].

$$\varepsilon = \frac{\Delta L}{L} \quad (2)$$

where:
ε – nominal strain;
L – initial distance between the grips [mm]; and
ΔL – increase in the distance between the grips after the test [mm].

Finally, the tensile modulus of each specimen was calculated based on the following formula (Equation 3):

$$E_t = \frac{\sigma_2 - \sigma_1}{\varepsilon_2 - \varepsilon_1} \quad (3)$$

where:
E_t – tensile modulus [MPa]; and
σ – stress [MPa] measured at the strain value $\varepsilon_1 = 0.0005$ and $\varepsilon_2 = 0.0025$.

The research was planned using the applicable ISO standards.[22,23] The minimal number of samples required, according to the norm for this type of research, is 5. In our study, the size of each group was $n = 10$.

Statistical analysis

The statistical analysis employed the Polish version of Statistica, v. 13 (StatSoft, Cracow, Poland). Since the results varied in the normality test, a non-parametric analysis of variance (ANOVA) test according to Kruskall–Wallis was used to compare the values ($p < 0.05$).

Results

Table 2 compares the basic statistics of the tensile modulus of the 3 presented resins. From the data shown, IBT differed the most from the other 2 resins (Dental LT Clear and BioMed Amber).

The homogeneity of variance test, presented in Table 3, revealed that some values (F, ΔL, σ, and ε) differed significantly when the 3 resins were compared. Welch's correction revealed even more differences, and the results are presented in Table 4.

Post-hoc tests were carried out to assess more detailed values, with Tukey's honestly significant difference (HSD) tests performed to compare pairs of materials. The results are shown in Tables 5–8. Almost all of the parameters were significantly different between the groups of materials. The lowest force required for damage occurred in

Table 2. Basic statistics of the tensile modulus of 3 dental materials

Young's modulus [MPa]	Material			*p*-value
	Dental LT Clear $n = 10$	BioMed Amber $n = 10$	IBT $n = 10$	
M ±*SD*	1.97 ±0.13	1.24 ±0.07	0.019 ±0.001	< 0.01*
Me (*IQR*)	1.96 (1.86–2.05)	1.24 (1.19–1.30)	0.019 (0.019–0.019)	
min–max	1.80–2.15	1.14–1.34	0.019–0.020	

M – mean; *SD* – standard deviation; *Me* – median; *IQR* – interquartile range; min – minimum; max – maximum; * statistically significant.

Table 3. Homogeneity of variance test according to Brown–Forsythe

Variable	SS effect	df effect	MS effect	SS error	df error	MS error	F	*p*-value
Mean height [mm]	0.000	2	0.000	0.006	28	0.0002	0.03740	0.963343
Mean width [mm]	0.000	2	0.000	0.021	28	0.0007	0.11910	0.888167
A [mm^2]	0.011	2	0.005	0.224	28	0.0080	0.67597	0.516767
F [N]	2,834.040*	2	1,417.020*	7,406.310*	28	264.5111*	5.35713*	0.010716*
ΔL [mm]	6.141*	2	3.070*	5.085*	28	0.1816	16.90864*	0.000015
L [mm]	0.000	2	0.000	0.000	28	0.0000	–	–
σ [MPa]	22.566*	2	11.283*	64.393*	28	2.2998	4.90626*	0.014904
ε	19.189*	2	9.595	15.888*	28	0.5674	16.90864*	0.000015

A – initial cross-sectional area; *F* – force; ΔL – increase in the distance between the grips after the test; *L* – initial distance between the grips; σ – tensile stress; ε – nominal strain; SS – sum of squares; df – degrees of freedom; MS – mean sum of sqares; * statistically significant.

Table 4. Variance analysis with Welch's correction

Variable	SS effect	df effect	MS effect	SS error	df error	MS error	F	*p*-value	df Welch effect	df Welch error	F (Welch)	*p*-value (Welch)
Mean height [mm]	0*	2	0*	0.01*	28	0.0004*	58.423*	0.00000*	2	17.78163*	55.904*	0.000000*
Mean width [mm]	0*	2	0*	0.04*	28	0.0015*	33.666*	0.00000*	2	18.18302*	27.073*	0.000004*
A [mm^2]	1*	2	0*	0.43*	28	0.0152*	29.630*	0.00000*	2	17.77987*	38.270*	0.000000*
F [N]	3,466,267*	2	1,733,134*	17,697.53*	28	632.0545*	2,742.063*	0.00000*	2	12.44415*	6,023.038*	0.000000*
ΔL [mm]	79*	2	40*	17.25*	28	0.6161*	64.175*	0.00000*	2	17.20851*	597.287*	0.000000*
L [mm]	–	2	0	0	28	0.0000	–	–	2	–	–	–
σ [MPa]	31,176*	2	15,588*	141.88*	28	5.0670*	3,076.380*	0.00000*	2	12.59515*	8,246.560*	0.000000*
ε	247*	2	124*	53.90*	28	1.9251*	64.175*	0.00000*	2	17.20851*	597.287*	0.000000*

* statistically significant.

the IBT tensile test, whereas Dental LT Clear showed the highest resistance to damage.

Non-parametric values were compared with the Kruskal–Wallis test. This referred to the initial cross-sectional area (*A*) of the probe. The obtained values (H) were not significantly different when Dental LT Clear and IBT were compared. However, the values were significantly different when either of the two materials was compared to BioMed Amber. The *Z*-value presents the standardization of the results, while the *p*-value refers to the significance of the obtained data. The data is presented in Table 9 and Table 10.

Table 5. HSD (honestly significant difference) – mean height [mm]

Pair comparison	Dental LT Clear (*M*: 1.9198)	BioMed Amber (*M*: 2.0182)	IBT (*M*: 1.9693)
Dental LT Clear	–	0.000125*	0.000144*
BioMed Amber	0.000125*	–	0.000148*
IBT	0.000144*	0.000148*	–

M – mean value of the assessed parameter; * statistically significant.

Table 6. HSD (honestly significant difference) – mean width [mm]

Pair comparison	Dental LT Clear (*M*: 5.3074)	BioMed Amber (*M*: 5.2264)	IBT (*M*: 5.1682)
Dental LT Clear	–	0.000317*	0.000125*
BioMed Amber	0.000317*	–	0.006543*
IBT	0.000125*	0.006543*	–

M – mean value of the assessed parameter; * statistically significant.

Table 7. HSD (honestly significant difference) – mean initial cross-sectional area (*A*) [mm^2]

Pair comparison	Dental LT Clear (*M*: 10.189)	BioMed Amber (*M*: 10.548)	IBT (*M*: 10.177)
Dental LT Clear	–	0.000125*	0.975750
BioMed Amber	0.000125*	–	0.000125*
IBT	0.975750	0.000125*	–

M – mean value of the assessed parameter; * statistically significant.

Table 8. HSD (honestly significant difference) – breaking force used (*F*) [N]

Pair comparison	Dental LT Clear (*M*: 437.25)	BioMed Amber (*M*: 830.35)	IBT (*M*: 17.718)
Dental LT Clear	–	0.000125*	0.000125*
BioMed Amber	0.000125*	–	0.000125*
IBT	0.000125*	0.000125*	–

M – mean value of the assessed parameter; * statistically significant.

Table 9. Kruskal–Wallis test for the initial cross-sectional area (*A*) [mm^2]

Pair comparison	Dental LT Clear (*R*: 11.100)	BioMed Amber (*R*: 25.800)	IBT (*R*: 11.545)
Dental LT Clear	–	0.000900*	1.000000
BioMed Amber	0.000900*	–	0.000999*
IBT	1.000000	0.000999*	–

H – obtained value (2; $N = 30$) = 17.16603; $p = 0.002$; * statistically significant.

Table 10. Kruskal–Wallis test for the initial cross-sectional area (*A*) [mm^2]

Pair comparison	Dental LT Clear (*R*: 11.100)	BioMed Amber (*R*: 25.800)	IBT (*R*: 11.545)
Dental LT Clear	–	3.615240*	0.112131
BioMed Amber	3.615240*	–	3.588185*
IBT	0.112131	3.588185*	–

H – obtained value (2; $N = 30$) = 17.16603; $p = 0.002$; * statistically significant.

Discussion

The present study of 10 samples of 3 selected materials (IBT, BioMed Amber and Dental LT Clear) met the ISO standards for this type of research,[22,23] and is in agreement with similar previously performed studies.[17,21] All 3 materials are dedicated for dental use, but have distinct characteristics. It means they are used for different purposes, which is supported by data on the mechanical features of these materials. Technically, all 3 materials could be used for surgical guides.[17,24] Dental LT Clear is willingly used for occlusal splints, e.g., in bruxism treatment.[25] Due to its perfect translucency and high esthetics, this transparent biomaterial could also be used in orthodontic treatment with clear aligners.[26] Although the manufacturers claim that IBT can be used for the production of surgical guides, it is primarily applied in the preparation of transfer trays, most often used for indirect bracket bonding in fixed-appliance techniques.[27] The presented tensile properties showed that IBT differed much from BioMed Amber, which is typically (according to the producer) used to prepare surgical guides. Since 3D printing is a developing branch of dentistry, there are not too many papers describing and comparing resins. Therefore, this paper is novel, and should provide more understanding of the nature and properties of resins.

BioMed Amber broke easily, though its dimensions did not change much. Although this material seems very promising, the resin is poorly described in the literature due to its novelty. Since the authors of the present study are aware of the limited amount of research on the properties of BioMed Amber, further investigations of the resin are planned. The current paper is part of a series presented by the authors.[17,21]

IBT proved to be the least resistant material. As presented in other studies, besides its original application in trays for indirect bracket bonding, IBT, due to its flexibility, could be used to produce mouth guards for protecting dentition during contact sports training, to prevent tooth trauma.[28] Other authors claim that the precision of trays for indirect bracket bonding does not depend on the material used, and is comparable when using resins from different manufacturers.[27] Other uses of this resin are disputable, and therefore research should be broadened. Although IBT Resin by Formlabs has not been thoroughly investigated, similar resins are described in the literature, as mentioned by the authors.

Dental LT Clear was the most resistant to damage; the force needed to break the sample was the highest. A comparison of Dental LT Clear and BioMed Amber in another study revealed that the tensile test situation was the opposite of the compression test outcome.[17] This material seems to raise great interest of other researchers, as several studies were conducted using it. Although Dental LT Clear was originally designed for occlusal splints and clear aligners, its other potential novel use was for a surgical tray in a Japanese study.[24] That study showed that the material could be more versatile, and the potential uses for Dental LT Clear resin could be widened.[24] Also, Dental LT Clear was tested for use in dental aligners; this specific resin shows high biocompatibility, but provides less precision as compared to a similar resin – Tera Harz.[29] Due to its biocompatibility and, after all, high precision, the material could be used for the preparation of individual appliances, such as those used in the rehabilitation of patients with clefts.[30] This aspect makes the resin worthy of further research.

Our study shows the advantages and disadvantages of three 3D-printable resins in terms of their mechanical features, and may be the key to a further investigation of potential resins uses. Dental LT Clear has also found its use in treating patients with cleft deformities, including those with the Pierre Robin sequence.[31] In the present study, the material showed the highest resistance to one type of external condition in the fracture load test. Although the above-mentioned research compared Dental LT Clear to other resins, it still required multiple printers, as the resins were produced by different companies.[31] Such a solution would be expensive for a technical laboratory or a dental office with regard to the equipment needed. Our study used 3 resins from the same manufacturer, so only one printer (Form 2 by Formlabs) was required. The examined resins displayed different properties and could be further investigated. Another study by the authors showed that samples which underwent compression testing differed in the texture and fractal dimension analyses.[32] In the compression test, the texture and fractal dimensions of Dental LT Clear did not change much,[32] which demonstrates that the material could be the most stable, as in the current study.

Although the research led to the formation of the presented conclusions, the authors are aware of some study limitations. The 1st limitation is that only 3 resins were analyzed, and they were chosen by the researchers. Another limitation might be the fact that the properties of the 3 chosen resins are different, which could cause difficulties in comparing the materials. However, the authors believe this is an advantage and could lead to further valuable research. The 3rd limitation is that the study was conducted in vitro, which is always the first step for this type of research. Nonetheless, the presented materials and methodology might be helpful in the examination of a higher number of specimens and materials in the future. The authors are also aware that the comparison of the material properties should be widened for the reasons outlined above.

Conclusions

In the tests performed on the IBT, BioMed Amber and Dental Clear LT materials, we found that:

- BioMed Amber Resin, tested for tensile strength, was the most repeatable; similar values of force were needed to break all of the resin specimens;
- the lowest tensile force was needed to cause damage to IBT Resin, which makes it the least stable and the least rigid resin tested;
- Dental LT Clear Resin was the most resistant to damage as compared to the other 2 resins, with the force needed to break the sample being the highest.

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

Digitalization era of dental education: A systematic review

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Abstract

Background. Dental education is taking its share of the digitalization of the world. Therefore, it is of value to assess the use of the digital dental education system, especially in the undergraduate period.

Objectives. This systematic review concisely evaluated the use of augmented and virtual reality in preclinical dental education.

Material and methods. The PICOS (Population, Intervention, Comparison, Outcome, and Study design) search strategy was used with the keywords 'e-learning', 'virtual reality' and 'preclinic simulation' to search the PubMed, PubMed Central, Web of Science, and Scopus databases.

Results. A total of 1,774 articles were found, and 45 articles were reviewed. The level of bias in the studies was also calculated. The studies were divided into 3 main groups: computer-assisted learning (C-AL); augmented reality-assisted learning (AR-AL); and virtual reality-assisted learning (VR-AL). Augmented and virtual reality are steadily evolving, and are increasingly being used in education and healthcare.

Conclusions. The evaluated technological applications enable the visualization of medical information and provide clear feedback during the learning process with increased security and reliability; thus, digital simulation systems can be used to enhance students' abilities in dentistry.

Keywords: augmented reality, virtual reality, computer-assisted learning, dental simulator, digital dentistry

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Introduction

Dentistry involves many skills, among which manual dexterity is the most difficult to maintain.[1–3] Dental students typically take a manual dexterity course during their first 2 years of education. As part of their preclinical practice, students often start with tasks involving simple geometric shapes rather than complex cavity or tooth preparations, and digital systems are rarely deployed. However, technological development is essential in modern dental practice. In addition, digital dental instruction shows high potential in facilitating both direct and distance learning in undergraduate and preclinical education. Indeed, applications based on three-dimensional (3D) imaging and printing, computer-aided design and computer-aided manufacturing (CAD/CAM),[4,5] augmented reality (AR), and virtual reality (VR) have been used in many fields, including dental research and practice, for over a decade.

Translational applications that use cameras in smartphones and popular face-swap applications are among the best-known AR applications encountered in everyday life. A well-known example of a VR application are the glasses used via game consoles. Applications of AR/VR are employed in various fields, such as entertainment, industry, medicine, and dentistry.[6–8]

Learning with digital technologies can be categorized as computer-assisted learning (C-AL), AR-assisted learning (AR-AL) or VR-assisted learning (VR-AL). Computer-assisted learning uses computer programs specially designed for education on specific topics. The AR-AL technology uses the existing reality or environments, and enriches them with a computer-generated scenario and the means of interaction. Meanwhile, the VR-AL technology uses only an artificial reality or environments, with which the user can interact.[9] By deploying these technologies, users have the opportunity to see preparations from different angles and at different magnifications. Moreover, the real-time feedback provided to the user permits consistent and standardized evaluation.[10]

The AR/VR components must be well-integrated to achieve the desired effect on users. These components include real and virtual data sources, tracking and registration techniques, visualization processes, perception locations, and feedback mechanisms.

In addition to the growing trend of deploying new information technology applications in education, the quarantine which came with the coronavirus disease 2019 (COVID-19) pandemic also had a marked impact on the education sector, where the traditional systems were forced to be replaced by digitized education. In many countries, students had to be quarantined at home for their safety and online education became the most popular solution for continuing their education, with accessibility and flexibility being among the most important criteria. The advantages of digital education have overcome the problems associated with the traditional education systems almost everywhere.[11] Online education has turned every place with access to the Internet into a classroom through personal terminals, such as computers, laptops and smartphones. Furthermore, the sudden closure of universities during the COVID-19 pandemic caused significant changes to the dentistry education system. Consequently, students began to think that they might either not succeed in graduating or graduate without having sufficient practical skills.[12] Due to such worries over their preclinical and clinical training adequacy in the context of distant learning, dental students struggled with elevated stress levels.[13–16]

If the education system is supported by the AR or VR technologies, students should receive distance education of a higher quality.[17,18] Even though it is well documented that dentistry education deploys the AR and VR technologies, education with AR or VR has never been evaluated along with C-AL systems. However, the C-AL technology should also be evaluated in the same scope as AR and VR.

The primary aim of this systematic review was to present the application areas for the C-AL, AR-AL and VR-AL technologies, and highlight their distinguishing features for dental education. Secondarily, it aimed to determine the application differences between these technologies within dental specialties. Thus, the present study was meant to establish a pathway for further research.

Methods

Ethics statement

This was not a human-subject study; therefore, neither approval by the institutional review board nor the obtainment of informed consent was required.

Protocol and registration

This systematic review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 guidelines. The research protocol was registered in the Open Science Framework (OSF) (https://doi.org/10.17605/OSF.IO/VTQAC).

Eligibility criteria

Table 1 outlines the inclusion and exclusion selection criteria details, which were based on the PICOS (Population, Intervention, Comparison, Outcome, and Study design) framework.

Table 1. Eligibility criteria according to the PICOS (Population, Intervention, Comparison, Outcome, and Study design) framework

PICOS items	Inclusion and exclusion criteria
Population	undergraduate dental students, students of dental specialties
Intervention	studies that explored the effect of the C-AL, AR-AL or VR-AL approaches, either alone or blended; e-learning intervention – web-based educational software, or special AR or VR devices studies not exploring the effects of the C-AL, AR-AL or VR-AL intervention were excluded
Comparison	studies with or without a comparison group
Outcome	studies that involved the investigation of learning outcomes related to knowledge content and clinical skills (cavity preparation, abutment preparation), as well as the assessment of students' knowledge; studies that explored learning outcomes related to students' attitudes, preferences and satisfaction from the learning activity
Study design	cross-sectional studies, case–control studies, cohort studies, observational trials, descriptive studies, and randomized controlled trials were included short communications, qualitative studies, commentary articles, letters to the editor, editorials, conference abstracts, book chapters, and reviews were excluded

C-AL – computer-assisted learning; AR – augmented reality; VR – virtual reality; AR-AL – AR-assisted learning; VR-AL – VR-assisted learning.

Information sources and the search strategy

A systematic electronic search of the PubMed, PubMed Central, Web of Science, and Scopus databases was performed. The search was limited to English-language articles published between January 1, 2000, and December 30, 2021.

The search syntax contained Medical Subject Headings (MeSH) terms, and the terms were as follows: (education, dental) AND (virtual reality OR virtual simulation) AND (e-learning OR electronic learning) AND (preclinical simulator OR phantom simulator). All abstracts were read, and duplicates and review articles were excluded. For the final stage, the full texts of all articles were read and relevant information was identified. Abstracts, short communications, letters to the editor, book chapters, and review articles were also excluded at this stage.

Selection process

Two authors (D.E. and B.G.) eliminated duplicated articles manually. They independently checked titles and abstracts to identify potentially eligible studies. If there was persistent disagreement between the 2 authors, a third reviewer (B.G.E.) made the final decision.

Data items and the data collection process

With regard to the general characteristics of the selected studies, the following data was extracted: authors; intervention design; type of digitalized learning technique; specialty; system used; participants; study design; and outcome measures. One author (D.E.) collected the data from the selected studies with the use of an extraction form prepared jointly by the authors. Another author (B.G.) verified the collected information. In instances of disagreement, a third author (B.G.E.) was consulted for the final decision.

Assessment of the risk of bias

The assessment of the risk of bias in the included studies was conducted independently by 2 authors (D.E. and B.G.), using the Medical Education Research Study Quality Instrument (MERSQI). The MERSQI is a tool for assessing the methodological quality of quantitative research articles. The scale consists of 10 items organized into 6 domains: study design; sampling; type of data; validity of the evaluation instrument; data analysis; and outcomes. The total score ranges from 5 to 18. The agreement between the 2 examiners' results was analyzed using the kappa (κ) statistical coefficient.

Synthesis methods

The data was classified and analyzed to achieve the objectives of this review. The study properties were extracted and key items, such as the acquisition of skills, the quality of preparations and feedback, were tabulated. Data synthesis was initially conducted by 2 authors (D.E. and B.G.), and then discussed with a third author (B.G.E.). The included studies were analyzed through a narrative synthesis. The types of learning methods and the specialization types are presented as percentages.

Assessment of the reporting bias

The reporting bias in this systematic review was independently assessed by 2 authors (D.E. and B.G.) for selective outcome reporting by comparing the study results with the previously published study protocols and registrations. Any disagreement was resolved by consulting a third author (B.G.E.).

Certainty assessment

Not done.

Results

Study selection

A total of 1,774 articles were retrieved via the electronic keyword search. Among these, 228 were evaluated further after eliminating duplicates. Finally, the review included 43 articles identified electronically, and an additional 2 articles were manually chosen from the reference lists of the other articles, resulting in a total of 45 articles (Fig. 1, Table 2).

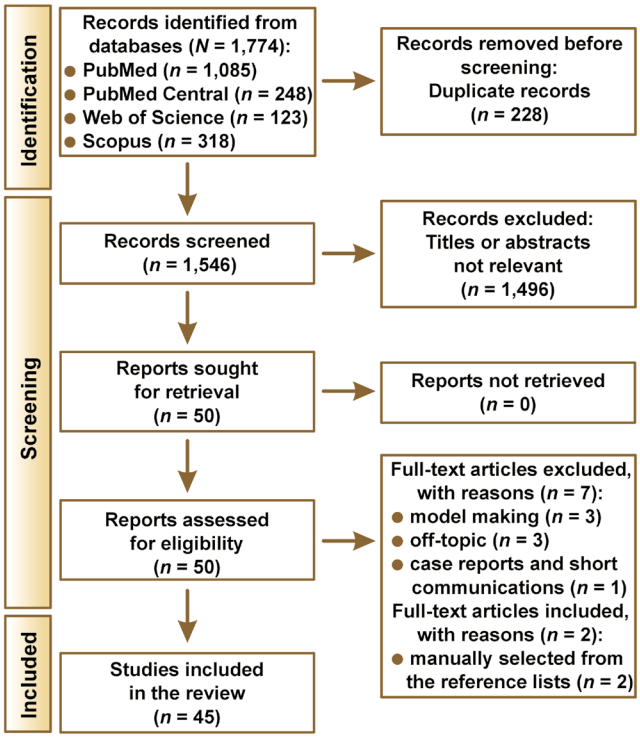

Fig. 1. Study selection process according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 guidelines

Study characteristics

The studies were divided into 3 main groups: C-AL (n = 5; 11%); AR-AL (n = 16; 36%); and VR-AL (n = 24; 53%). Most articles could be categorized into more than one group, so classification was based on the primary objective of the study (Table 1).

Risk of bias in the studies

The MERSQI scale assessed the methodological quality of half of the included studies as relatively moderate, with a mean score of 10.72 ±1.52 (median (interquartile range) (*Me* (*IQR*)): 11.5 (6.0–12.5)) (Table 3). The kappa coefficient of concordance was 0.74.

Results of individual studies

Relevant data from the included studies are grouped and summarized separately in Table 4.

Synthesis results

All the programs used in the C-AL studies were produced for experimental purposes. Except for one study,[49] all studies reported that C-AL improved students' education and satisfaction (9%).[38,44,46,50]

Many studies stated that VR-AL-based education is as effective as the traditional methods (33%).[29,31,33,34,40,43,47,51–55,58,62,63] However, a few studies reported that VR education was not sufficient and needed improvement (9%).[23,36,42,48] One study reported that educating with the use of VR alone was not suitable for undergraduate education.[19]

Although few studies examined AR-AL systems, many reported that they were effective for pregraduate education (24%).[22,25,26,28,32,35,37,39,41,57,61] Meanwhile, only one study reported no difference in success between the traditional and AR-AL-based education.[24]

Table 2. Studies excluded after full-text consideration with the corresponding main reason for exclusion

Study	Main reason for exclusion
Razavi M, Talebi HA, Zareinejad M, Dehghan MR. A GPU-implemented physics-based haptic simulator of tooth drilling. *Int J Med Robot*. 2015;11(4):476–485. doi:10.1002/rcs.1635	model making
Höhne C, Schmitter M. 3D printed teeth for the preclinical education of dental students. *J Dent Educ*. 2019:83(9):1100–1106. doi:10.21815/JDE.019.103	model making
Hanisch M, Kroeger E, Dekiff M, Timme M, Kleinheinz J, Dirksen D. 3D-printed surgical training model based on real patient situations for dental education. *Int J Environ Res Public Health*. 2020;17(8):2901. doi:10.3390/ijerph17082901	model making
Marras I, Nikolaidis N, Mikrogeorgis G, Lyroudia K, Pitas I. A virtual system for cavity preparation in endodontics. *J Dent Educ*. 2008;72(4):494–502. PMID:18381855.	off-topic
Saxena P, Gupta SK, Mehrotra D, et al. Assessment of digital literacy and use of smart phones among Central Indian dental students. *J Oral Biol Craniofac Res*. 2018;8(1):40–43. doi:10.1016/j.jobcr.2017.10.001	off-topic
Botelho MG, Gao X, Jagannathan N. A qualitative analysis of students' perceptions of videos to support learning in a psychomotor skills course. *Eur J Dent Educ*. 2019;23(1):20–27. doi:10.1111/eje.12373	off-topic
Padilla M, Nocera L, Abe Y, Clark GT. A modern web-based virtual learning environment for use in dental education. *J Dent Educ*. 2020. doi:10.1002/jdd.12427	short communication

Table 3. Assessment of the methodological quality of the included studies using the Medical Education Research Study Quality Instrument (MERSQI)

Study	Study design	Sampling		Type of data	Validity of the evaluation instrument			Data analysis		Outcomes				Total score
		institution studied	response rate score		internal structure	content	relationships with other variables	appropriateness of analysis	complexity of analysis	attitudes, perceptions, satisfaction	knowledge, skills	behaviors	pateient/healthcare outcome	
Quinn et al.[19]	3	0.5	1.5	1	0	1	0	1	2	1	1.5	0	0	12.5
Quinn et al.[20]	3	0.5	1.5	1	0	1	0	1	2	1	1.5	0	0	12.5
Imber et al.[21]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
LeBlanc et al.[22]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
Heiland et al.[23]	1	0.5	1.5	1	0	1	0	1	0	0	0	0	0	6
Jasinevicius et al.[24]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
Wierinck et al.[25]	1.5	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	10
Wierinck et al.[26]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
Wierinck et al.[27]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
Rees et al.[28]	1	0.5	1.5	1	0	1	0	1	1	0	1.5	0	0	8.5
Von Sternberg et al.[29]	2	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	10.5
Wierinck et al.[30]	3	0.5	1.5	1	0	1	1	1	2	0	1.5	0	0	12.5
Suebnukarn et al.[31]	2	0.5	1.5	1	0	1	1	1	2	0	1.5	0	0	11.5
Urbankova[32]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
Pohlenz et al.[33]	1	0.5	1.5	1	0	1	0	1	1	1	0	0	0	8
Suebnukarn et al.[34]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
Urbankova and Engebretson[35]	1	0.5	1.5	1	0	1	0	1	1	0	1.5	0	0	8.5
Gal et al.[36]	2	0.5	1.5	1	0	1	1	1	1	1	0	0	0	10
Urbankova and Engebretson[37]	1	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	9.5
Woelber et al.[38]	1	0.5	1.5	1	0	1	0	1	2	1	1.5	0	0	10.5
Tanzawa et al.[39]	1	0.5	1.5	1	0	1	0	1	1	1	0	0	0	8
Urbankova et al.[40]	1	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	9.5
Kikuchi et al.[41]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
Ben-Gal et al.[42]	2	0.5	1.5	1	0	1	1	1	2	0	1.5	0	0	11.5
Yamaguchi et al.[43]	1	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	9.5
Moazami et al.[44]	1	0.5	1.5	1	0	1	0	1	1	0	1.5	0	0	8.5
Eve et al.[45]	3	0.5	1.5	1	0	1	1	1	2	1	1.5	0	0	13.5
Reissmann et al.[46]	1	0.5	1.5	1	0	1	1	1	2	1	0	0	0	10
Wang et al.[47]	1	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	9.5
Koo et al.[48]	3	0.5	1.5	1	0	1	0	1	2	1	1.5	0	0	12.5
Mehta et al.[49]	3	0.5	1.5	1	0	1	0	1	2	1	0	0	0	11
Ludwig et al.[50]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
De Boer et al.[51]	3	0.5	1.5	1	0	1	0	1	2	1	0	0	0	11
Al-Saud et al.[52]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
De Boer et al.[53]	1	0.5	1.5	1	0	1	0	1	2	1	1.5	0	0	10.5
Mirghani et al.[54]	2	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	10.5
Dwisaptarini et al.[55]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
Ria et al.[56]	2	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	10.5
Llena et al.[57]	3	0.5	1.5	1	0	1	0	1	2	1	1.5	0	0	12.5
De Boer et al.[58]	3	0.5	1.5	1	0	1	0	1	2	1	1.5	0	0	12.5
Murbay et al.[59]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
Vincent et al.[60]	3	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	11.5
Tang et al.[61]	3	0.5	1.5	1	0	1	0	1	2	1	1.5	0	0	12.5
Zafar et al.[62]	1	0.5	1.5	1	0	1	0	1	2	1	0	0	0	9
Aliaga et al.[63]	1.5	0.5	1.5	1	0	1	0	1	2	0	1.5	0	0	10

Table 4. Studies investigating the use of digitalized learning techniques for acquiring new skills in education

Authors	Intervention design	Type	Specialty	System used	Participants	Study design	Outcome measures
Quinn et al.[19]	split cohort	VR-AL	restorative	experimental	20 students	instructor feedback (conventional) vs. real-time feedback (conventional) vs. real-time feedback (VR)	VR-based skill acquisition is unsuitable for use as the sole method of feedback and evaluation for novice students
Quinn et al.[20]	split cohort	VR-AL	restorative	experimental	32 students	instructor feedback (conventional) vs. real-time feedback (VR)	the VR group performed better for outline, depth and smoothness
Imber et al.[21]	cohort	AR-AL	restorative	DentSim	26 students	the scores achieved by each student in the last 6 simulator cavities were compared to their final comprehensive grades	simulation exercises are an efficient way to allow the early identification of those who are likely to perform poorly
LeBlanc et al.[22]	split cohort	AR-AL	restorative	DentSim	68 students	the comparison of practical skills in the traditional preclinical laboratory or in combination with a VR simulator	training for 6–10 h improved students' grades
Heiland et al.[23]	cohort	VR-AL	surgery	VOXEL-MAN	40 students	the evaluation of the new teaching modality was performed with the help of a questionnaire using ranking scales	further development to extend the range of simulated surgical procedures
Jasinevicius et al.[24]	split cohort	AR-AL	restorative	DentSim	28 students	3 categories covering both CS and VR	no statistical differences were observed in the quality of preparations; the instruction time was reduced in the VR group
Wierinck et al.[25]	split cohort	AR-AL	restorative	DentSim	42 students	skill acquisition with CS or VR	the feedback group showed higher grades
Wierinck et al.[26]	split cohort	AR-AL	restorative	DentSim	36 students	CS with instructions, VR with feedback, and VR with instructions and feedback	simulation with instructions and feedback showed better performance after 4 months
Wierinck et al.[27]	split cohort	AR-AL	restorative	DentSim	36 students	CS vs. VR with semi- or full feedback	no difference was observed between semi- or full feedback in simulation
Rees et al.[28]	split cohort	AR-AL	restorative	DentSim	16 students	the critical appraisal of the software by students, and the correlation of the preparation time with the final score and the number of evaluations	the positive evaluation of the software Class II cavities was associated with a longer preparation time, and a longer preparation time was associated with higher scores
Von Sternberg et al.[29]	split cohort	VR-AL	surgery	VOXEL-MAN	41 students	tested whether the skills acquired on a virtual apicectomy simulator are transferable from virtual to physical reality	training with a virtual apicectomy simulator appears to be effective and the acquired skills are transferable to physical reality
Wierinck et al.[30]	cohort	AR-AL	restorative	DentSim	6 students, 12 specialists	a restorative dentist, a periodontist and a novice student prepared cavities	it was possible to distinguish experts in operative dentistry from experts in periodontology
Suebnukarn et al.[31]	split cohort	VR-AL	prosthodontics	experimental	10 students 10 residents	a crown preparation task with a haptic VR system that provided FFB to the operating tool was performed while interacting with the virtual tissue/organ	novice and expert performance can be demonstrated in crown preparation by using a haptic VR system
Urbankova[32]	split cohort	AR-AL	restorative	DentSim	79 students	students were randomized to CDS training or the traditional preclinical dental training alone	8 h of CDS training administered early in the preclinical operative dentistry course may improve student performance
Pohlenz et al.[33]	cohort	VR-AL	surgery	VOXEL-MAN	53 students	assessed the realism of a VR system for dental applications	the haptic simulator was considered suitable for training purposes in surgical endodontics by 51 students
Suebnukarn et al.[34]	split cohort	VR-AL	endodontics	experimental	32 students	students were randomly assigned to train on either micro-CT tooth models with a haptic VR simulator or extracted teeth in a phantom head training environment for 3 days, after which the assessment was repeated	training on the haptic VR simulator and the conventional phantom head had equivalent effects on minimizing procedural errors in endodontic access cavity preparation

Authors	Intervention design	Type	Specialty	System used	Participants	Study design	Outcome measures
Urbankova and Engebretson[35]	cohort	AR-AL	restorative	DentSim	38 students	cavity preparations during a single 4-hour CDS pre-test prior to the operative dentistry course and during the subsequent practical examinations	a pre-course CDS test may help to identify students in need of early instructional intervention
Gal et al.[36]	split cohort	VR-AL	prosthodontics	IDEA	21 educators, 12 students	participants performed drilling tasks using a simulator, and filled out a questionnaire regarding the simulator and the potential ways of using it in dental education	the development of the simulator's tactile sensation is needed to attune it to genuine sensation
Urbankova and Engebretson[37]	split cohort	AR-AL	restorative	IDEA	39 students	tested whether an AR pre-test can predict preclinical operative dentistry examination scores	a pre-course CDS test may help to identify students in need of early instructional intervention
Woelber et al.[38]	split cohort	C-AL	periodontology	experimental	85 students	one group studied with a laborious, high-interaction e-learning program, while the other group studied with a low-interaction learning environment	e-learning programs for case-based learning do not have to be overly laborious to be useful
Tanzawa et al.[39]	cohort	AR-AL	restorative	robot patient	88 students	the efficiency of a robot patient in education	the importance of using such a robot in education was stressed
Urbankova et al.[40]	cohort	VR-AL	restorative	IDEA	39 students	the association of haptic VR simulator exercise with preclinical dentistry practical exam scores	complex haptic exercise was strongly associated with early student preclinical performance
Kikuchi et al.[41]	split cohort	AR-AL	prosthodontics	DentSim	43 students	group 1 received device-only feedback, group 2 received instructor feedback and group 3 received no feedback	the AR system improved student training for PFM crown preparation.
Ben-Gal et al.[42]	split cohort	VR-AL	geometric shapes	IDEA	63 students, 28 dentists, 14 non-dentists	performed virtual drilling tasks in different geometric shapes	improved construct validity, a shorter working time and more difficult tasks should be introduced
Yamaguchi et al.[43]	cohort	VR-AL	restorative	VR simulation	7 students	the evaluation of haptic VR simulation with repetitive training as a tool in teaching caries removal and periodontal pocket probing skills	VR simulation was effective in the acquisition of hand skills for caries removal
Moazami et al.[44]	split cohort	C-AL	endodontics	experimental	40 students	experimental (virtual) and comparative (traditional) learning were evaluated	the comparison of the mean knowledge scores for both groups showed that virtual learning was more effective than the traditional learning
Eve et al.[45]	split cohort	VR-AL	restorative	Virteasy	12 students, 14 residents	compared the performance of students vs. residents	residents removed greater amounts of carious and sound tissue
Reissmann et al.[46]	split cohort	C-AL	prosthodontics	OLAT	71 students	students participated in a course with blended learning content; for comparisons, data was obtained from 2 courses in the previous years and 3 courses in the subsequent years	the e-learning tool was appreciated by students, suggesting that learning objective tests can be successfully implemented in blended learning
Wang et al.[47]	split cohort	VR-AL	restorative and endodontics	iDental	10 residents, 10 dentists	the evaluation included 2 dental drilling tasks – a caries removal operation and a pulp chamber opening operation	no significant differences could be found between the 2 groups, and the volume of removed caries and the depth of pulp chamber insertion showed small standard deviations
Koo et al.[48]	split cohort	VR-AL	restorative	IDEA	34 students	assessed the perception of haptic-based manual dexterity training	the manual dexterity module software was not superior in improving dexterity
Mehta et al.[49]	split cohort	C-AL	orthodontics	experimental	63 students	32 students received electronic access to e-learning material covering various undergraduate orthodontic topics over a 6-week period, while 31 control students were not given the access during the study period	the use of the novel orthodontic e-resource by fourth-year undergraduate students over a 6-week period did not result in significant improvement in subject knowledge

Authors	Intervention design	Type	Specialty	System used	Participants	Study design	Outcome measures
Ludwig et al.[50]	split cohort	C-AL	orthodontics	experimental	30 students	10 students underwent a specifically designed program based on a PPT, other students underwent a commercially available program and 10 students served as controls	blended learning produced better learning outcomes as compared to the use of the traditional teaching method alone; the easy-to-use PPT-based custom software produced better results than the commercially available software
De Boer et al.[51]	split cohort	VR-AL	geometric shapes	Simodont	124 students	2D vs. 3D preparations	3D vision has a positive effect on student performance
Al-Saud et al.[52]	split cohort	VR-AL	geometric shapes	Simodont	63 non-dentists	group 1 received device-only feedback, group 2 received verbal feedback from a qualified dental instructor, and group 3 received a combination of instructor and device feedback	the acquisition and retention of basic dental motor skills in novice trainees is best optimized through a combination of instructor and visual display (VR)-driven feedback
De Boer et al.[53]	split cohort	VR-AL	geometric shapes	Simodont	101 students	practiced with or without FFB	FFB is important for performance in a VR environment
Mirghani et al.[54]	split cohort	VR-AL	geometric shapes	Simodont	289 students	examined the sensitivity of a haptic VR dental simulator to differences in dental training experience	statistically significant differences were found between novice and experienced students
Dwisaptarini et al.[55]	split cohort	VR-AL	restorative	experimental	32 students	students were randomly assigned to train on either a simulator or conventional extracted teeth for 3 days, after which the assessment was repeated	the VR simulator and the conventional tooth practice had equivalent effects on improving performance in minimally invasive caries removal
Ria et al.[56]	cohort	VR-AL	restorative	hapTEL	101 students	to assess the learning progression of novice dental students using haptic virtual workstations	the system improved student performance in simulated cavity preparation
Llena et al.[57]	split cohort	AR-AL	restorative	AR	41 students	the traditional teaching methods vs. AR	the AR techniques favored the acquisition of knowledge and skills, and were regarded as a useful tool by students
De Boer et al.[58]	split cohort	VR-AL	geometric shapes	Simodont	126 students	a manual dexterity test in a VR environment with automatic assessment after a 3-month period of practicing with standard FFB	after practice for a sufficient amount of time at one level of FFB, the skill was transferable from one level of FFB to another
Murbay et al.[59]	split cohort	VR-AL	restorative	Simodont	32 students	exposure to VR vs. no exposure to VR	the use of VR significantly improved the satisfactory performance of students
Vincent et al.[60]	split cohort	VR-AL	restorative	Virteasy	88 students	cavity preparations with VR or conventional simulators	VR allowed assessment based on objective criteria and reduced subjectivity
Tang et al.[61]	split Cohort	AR-AL	prosthodontics	DCARER	60 students	traditional vs. digitalized tooth preparation	the tooth preparations of the traditional group scored significantly lower than those of the digital group
Zafar et al.[62]	cohort	VR-AL	pediatric dentistry	Simodont	100 students	the perception of the dentistry training gained in VR and a conventional simulation environment	VR could be used as an adjunct in training dental students for pre-clinical pediatric dentistry restorative exercises
Aliaga et al.[63]	cohort	VR-AL	restorative	Simodont	82 students	the methacrylate block practice criteria and the evaluation scale were assessed in the 1st and 3rd years	both methodologies can detect manual skill improvement in dental students; additionally, the Simodont practice can be reliably evaluated

IDEA – Individual Dental Education Assistant; CS – contemporary non-computer-assisted simulation system; FFB – force feedback; CDS – computerized dental simulator; CT – computed tomography; PPT – PowerPoint presentation; 2D – two-dimensional; PFM – porcelain-fused-to-metal.

AR-AL systems were mainly used for restorative dentistry training (29%),[21,22,24–28,30,32,35,37,39,57] more so than VR-AL systems (27%),[19,20,40,43,45,47,48,55,56,59,60,63] and no study related to restorative dentistry teaching used a C-AL system. One study (2%) used C-AL for endodontics training,[44] while 2 (4%) used VR-AL systems for this purpose.[34,47] All orthodontics training studies (4%) employed a C-AL system.[49,50] Only one study on pediatric dentistry training (2%) used a VR-AL system[62] and 1 (2%) studied periodontology training with a C-AL system.[38] Two AR-AL system studies (4%),[41,61] 2 VR-AL system studies (4%)[31,36] and 1 C-AL system study (2%)[46] were found for prosthodontics. All 3 surgery studies (6%) used VR-AL systems.[23,29,33]

All 6 studies with a geometric shape subject area that could not be attributed to any dental specialty (13%) employed VR-AL systems.[42,51,52–54,58]

Reporting bias

The search showed that none of the protocols or records of the included studies were previously registered. As such, the risk of reporting bias was unclear, as it was not possible to determine if all results were included in the published reports.

Discussion

Modern digital technologies have been used in medical specialties, such as surgery and echocardiography, for decades.[64] The VR and AR systems used in neurosurgery and cranial surgery are highly advanced, although they are not as common in dentistry.[65] Surgeons can access information on the patient's medical status throughout the operation by using smart glasses.[66] Such features may play a vital role in reducing surgical risks, even during routine procedures.

New technologies have recently become available for medical and dental education. While some of these technologies let the lecturer communicate with students, other digital technologies, such as cloud-based systems, allow data storage and its timely access. Therefore, using digital technologies for education enables low-cost, easy-to-use, reproducible, and equitable assessment and evaluation of students.[67] Among the types of digitalized learning, Internet-based and electronic educational applications fall within the scope of e-learning.

C-AL applications are programs that proceed within the framework of specific algorithms and contain codes specially written for education, though they do not include any real or virtual practical application steps. AR-AL uses a computer to process the data collected during the dental operation, and provides instructions and feedback to the user. VR-AL allows the operator to use haptic devices for dental procedures in a completely virtual environment, with instructions and feedback received from the computer.

Computer-assisted learning, augmented reality-assisted learning and virtual reality-assisted learning

Learning has been defined functionally as changes in behavior that result from experience, or mechanistically as changes in the organism that result from experience.[68] In a typical classroom setting, information generally flows in only one direction, which can be considered passive learning. However, current education requires the development of more active learning through interactive systems. This transformation in learning has already started and has become a critical factor in health education. For example, anatomy is one of the specialties that has benefited most from AR; consequently, it is now possible to learn live anatomy by visualizing internal structures.[69]

Many studies have assessed C-AL in dentistry, especially during undergraduate education. With the use of C-AL, the educational satisfaction level of students increases.[44] Including interactive tasks in the curriculum may also prevent demotivation in students.[38] Therefore, using C-AL may be an efficient way to enhance learning, especially during repetitive tasks.[50] Another benefit of using C-AL is that it requires less hardware than AR-AL and VR-AL systems.

During preclinical dental education, students receive directions from instructors, who evaluate their work before they proceed. Students generally receive feedback directly from instructors at the end of the procedure. However, such delayed feedback may potentially lead to overlooking errors. With VR-AL, students learn faster and perform a higher number of exercises as compared to the traditional systems, and also receive regular internal evaluation, which reduces the time needed for the assessments made by instructors.[70]

Some authors have reported that VR-assisted training is not a sufficient replacement for the traditional training.[62] Indeed, only 27% of the participating students found the textural or tactile sensation to be adequately close to reality.[62] Generally, the traditional educational methods are used alongside supportive digital educational methods. Therefore, including standardized digital learning materials with real-time feedback would increase student performance and reduce the learning time. Such an approach would allow students to practice more repetitive procedures for the development of motor skills.

Skill acquisition using augmented and virtual reality systems

Manual dexterity is used to execute motor skills, and is a permanently acquired ability resulting from practice and experience. The skills associated with AR and VR are continually evolving, and are becoming increasingly used in education and healthcare. These technological applications are useful, as they allow the precise visualization

of medical information. Moreover, these systems provide more accurate information during the process, resulting in increased security and reliability.[71] By applying the same principles to restorative dentistry, AR and VR simulations can help students improve manual dexterity during Class I and Class II cavity preparations.[57]

Although this systematic review included only 45 studies, many articles describe the future of digitalized dentistry applications. Both AR and VR systems play a substantial role in dental education. Moreover, AR systems could be used as an educational standardization medium. One of the studies investigated and compared the removal of carious lesions by novice students and experienced dental residents, and found that novice students removed less material, including sound and carious tooth structure, than residents; the results also demonstrated that caries removal skills could be taught by using computer simulators.[45]

Immediate feedback is one of the major advantages of digital systems, with many using application time data, target-based data, clinical step data, and motion and force exertion tracking data to provide feedback to the user. Digital simulators can provide information on the preparation size and the amount of removed matter, and observe the stroke magnitude and the movement speed, thereby monitoring the user's professionalism.[72] It has been suggested that just an additional 8 h of computer simulation can improve learning performance.[32] A similar study also reported that increasing computer simulation instruction from 6 to 8 h improved practical exam grades among second-year dentistry students.[22] With the help of this technology in dental education practical training, it is possible to simulate different scenarios and provide feedback on student performance. In particular, the haptic devices used in VR provide results comparable to a real environment.[73] Therefore, learning can be enhanced more easily with VR-AL systems than AR-AL systems.

Another application of AR is a mapping scheme called 'seeing through reality', which can be used by operators during surgery.[74] Such systems not only assist with operations, but also supply navigation and guidance in real time during surgery, specifically in implant dentistry.[75] The AR technology also has diagnostic and treatment planning applications. Navident is the latest AR-assisted implant surgery system. Furthermore, AR-assisted guided surgery is more precise. Even in risky anatomic zones, the average error of the system is approx. 0.96 ±0.7 mm.[76]

Using digital interfaces in dental education has some limitations. Continuous feedback may cause practitioners to become overly reliant on the system and the cessation of feedback may subsequently lower their performance.[77] Although feedback is essential for improvement, reducing feedback frequency facilitates the development of motor and cognitive skills.[78] The cognitive load theory predicts that learning varies with feedback during practice. Reduced feedback necessitates more planning to execute the task, whereas increased feedback can cause an information overload.[77,79]

Distance learning with augmented and virtual reality systems

Although it is challenging to execute practical exercises remotely, distance learning has become a requirement in education, especially due to the concerns regarding face-to-face learning during the COVID-19 pandemic. Nevertheless, there are some disadvantages associated with distance (or online) learning, such as isolation from the community, which can cause participants' discomfort and reduce motivation for learning.[80] In particular, using webcams and microphones can reduce course participation, and cause distractions or difficulties in focusing.

AR glasses, or smart glasses (e.g., HoloLens 2, Meta Quest 2 and Google Glass), show promising effects in education and medicine, and can be an alternative tool in distance learning. AR glasses involve 3 forms of interaction: gaze; gesture; and voice. Additionally, AR glasses can use the position-tracking technology to locate and track the user in their 3D environment, and is equipped with the orientation-tracking technology to recognize what the user is looking at.

Some programs work with AR glasses in an educational context, including HoloHuman and HoloPatient.[81] It has been reported that 68% of students agree that the dentistry curriculum statement must include HoloHuman as a supplementary teaching tool during anatomy lectures.[82] Another program, HoloDentist, connects 2 distant dentists or students to enable communication and the exchange of information. The newly developed portable learning platform DenTeach consists of smart sensors, advanced robotics, big data handling, 3D printing, AR, and cloud-based computing. This platform is applicable to distance learning in preclinical education.[83]

AR and VR systems contain processors, software, sensors, and input parts that work together. One limitation of the AR and VR technologies is that development can occur only within the framework of the infrastructure, i.e., software updates can only be performed if the system hardware configuration has the necessary permit. Thus, AR systems, which use a real environment, can be more advantageous in terms of visualization quality than VR systems.

The digital technologies used in dentistry education have both advantages and disadvantages. While C-AL does not contribute adequately to the development of motor skills, it provides an advantage in distance education and reduces educational costs.[38,44,46,49,50] AR systems are advantageous for developing motor skills, since the physical environment used is very close to a real environment, although they do have disadvantages as well, such as the need for consumables, which increase training costs.[21,22,25–28,32,37,39,41,57,61] While VR systems enhance motor skills without requiring consumables, they need to be developed to imitate a real environment.[19,20,23,29,33,34,36,40,43,47,52,53,56,58–60,62] With a reduction

in the cost of haptic parts, AR systems are a promising technology for distance education in the future. Since all digital systems provide objective criteria for evaluating the user, they should be included in dental training.

It should be emphasized that our research was limited to articles related to software developed for educational purposes only. Programs related to dentistry that may also be used for educational purposes in the future were not included. Despite the attempt to include as many accessible studies as possible, only a limited number of them were found to be relevant to this study. Furthermore, considering how rapidly technology is evolving and blended learning is becoming a modular and adaptable teaching and learning approach, it is probable that there exist publications which would either support or contradict the findings of this review. Another limitation of the study is the exclusion of non-English articles.

Future research is needed to explore the feasibility of digital technologies in these areas. Furthermore, evidence for the long-term effect of C/AR/VR-assisted training on student clinical performance and competence, as well as data regarding the cost-effectiveness of these devices, are currently lacking.

Conclusions

In the current era, with digital technologies being frequently used in all areas, it has become necessary to use them to improve students' skills. Among these digital technologies, C-AL, AR-AL and VR-AL were the focus of this study. One of the main advantages of AR-AL and VR-AL systems is that they facilitate manual skill acquisition and provide instant feedback. When considering C-AL systems, even though they are proficient in knowledge transfer, they are inferior as compared to AR-AL and VR-AL systems in terms of manual skill acquisition. The biggest feature distinguishing AR-AL systems from VR-AL systems in education is that in the former case there is no disconnection from reality. AR-AL gives students the feeling of being close to a real environment, provides information on the current situation and guides them during dental treatment. Nevertheless, C-AL, AR-AL and VR-AL applications cannot be considered adequate replacements for the traditional preclinical instruction. While distance education is possible, we believe that the instructor and the student must be physically present in the same setting for learning to be most effective. Even though C-AL, AR-AL and VR-AL applications can be easily implemented into dental education, further studies are needed to elucidate the benefits of these emerging digital technologies to the learning processes.

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

Systematic review of preventive and treatment measures regarding orthodontically induced white spot lesions

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Abstract

White spot lesions (WSLs) are one of the most common adverse effects following comprehensive fixed orthodontic treatment. The purpose of this review was to evaluate recent studies addressing the prevention and treatment of these lesions. Electronic databases were searched for English-written studies published between 2015 and October 2020 involving randomized clinical trials aiming at prevention or treatment of orthodontically induced WSLs using the following keywords in their title or abstracts: randomized clinical trial OR randomized controlled trial AND white spot OR caries OR demineralization OR decalcification OR remineralization. From the 23 papers which met the inclusion criteria, 11 were on preventive methods, while 12 addressed treatment protocols. However, most of the reviewed studies had a high risk of bias. The results of this review strongly support the importance of oral hygiene observation in preventing WSLs. Sodium fluoride varnish 5% was confirmed to be effective in the treatment of these lesions, as well as in the prevention of WSLs in patients with suboptimal oral hygiene. In addition, immediate CO2 laser irradiation after bonding can effectively prohibit formation of WSLs during orthodontic treatment. The literature also illustrates a promising masking effect of resin infiltration for the treatment of WSLs. However, little scientific evidence supports the effectiveness of Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) against WSLs, although more clinical trials with long-term follow-up are needed. Oral hygiene maintenance is crucial in the prevention of WSLs, and 5% sodium fluoride varnish and CO2 laser irradiation are recommended in patients with compromised oral hygiene. In the case of WSL formation, fluoride varnish and resin infiltration are effective treatment modalities.

Keywords: dental caries, tooth remineralization, fixed orthodontic appliances, randomized controlled trial

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Introduction

A common and unpleasant consequence of fixed orthodontic treatments is the development of white spot lesions (WSLs). These are characterized by white opaque lesions that are formed as a result of increased enamel subsurface porosities, which are early and reversible caries.[1–3] While regular caries take at least 6 months to develop, WSLs may become visible within a month following bonding of attachments.[4]

Since one of the main goals of orthodontic treatment is to improve smile esthetics, WSLs can negatively affect treatment outcomes and lead to patient dissatisfaction,[5] and they have a prevalence of 4.9 to 84%. This wide range of prevalence could be attributed to different study designs and the variety of methods used for detection and quantification of demineralized areas.[6] Although almost every tooth can be affected, most of the studies report higher involvement rates in the lateral incisors.[7–9]

Improvement of WSLs appearance mostly occurs in the first 6-24 months after deboning. This is due to three major factors: oral hygiene improvement and reduction in Streptococcus mutans and Lactobacillus spp to baseline level, remineralization using fluoride-containing dentifrices and mouth rinses, and abrasion of lesions due to brushing.[10,11]

Several interventions have been introduced for WSL management. Some are based on remineralization using high concentration fluoride components such as varnish, mouth rinse, and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) (MI paste/ MI paste plus), while others focus on improvement of appearance by other methods like bleaching, micro-abrasion, and resin infiltration.[12,13]

The aim of this literature review was to investigate the latest randomized clinical trials in order to clarify evidenced-based measures for prevention and treatment of WSLs during and after fixed orthodontic treatment.

Methods

Protocol and registration

The current systematic review followed the PRISMA guidelines[14] and the Cochrane Handbook for the Systematic Review of Interventions (version 5.1.0).[15]

Eligibility criteria

According to the PICOS format (Table 1), we included randomized controlled trials on human patients comparing various preventive/therapeutic measures addressing orthodontically induced WSLs to a control/placebo group.

Table 1. PICO format of the review

Population	Patients with fixed orthodontic treatment, with post orthodontic white spot lesions
Intervention	Various preventive/therapeutic measures for WSLs (except veneers)
Comparison	No intervention, normal home care
Outcome	Effects on mineral content, appearance, or extent of lesions

The eligibility criteria are summarized below.

Inclusion criteria:

– randomized clinical trial studies;
– treatment by conventional fixed orthodontic appliances;
– at least one post-orthodontic WSL (trials concerning WSL treatment)
– studies investigating different preventive and therapeutic strategies except veneers; and
– papers published in English between 2015 and October 2020.

Exclusion criteria:

– other designs of studies;
– animal studies and in vitro studies; and
– WSLs not stated to be the consequence of orthodontic treatment

Information sources and search strategy

A comprehensive electronic search was performed in PubMed, Scopus, and Embase databases from 2015 to October 2020 using the following keywords in their title or abstracts: randomized clinical trial OR randomized controlled trial AND white spot OR caries OR demineralization OR decalcification OR remineralization (Table 2). We manually added suitable articles for inclusion, and the reference lists were screened for new trials.

Study selection and data extraction

A total of 1924 papers were identified through the electronic search. After duplicate papers were removed by EndNote X9, 909 papers remained. Afterwards, two investigators independently evaluated the titles and abstracts considering the inclusion and exclusion criteria. Inconsistencies between reviewers were excluded from the study unless a consensus was reached. Full texts of 28 relevant papers were selected and reviewed thoroughly, and 23 articles met the final criteria for entering this review (Figure 1). We extracted the following data from the included studies to facilitate comparison among the articles: number of participants, type of intervention, follow-up time, diagnostic method used, and conclusions.

Table 2. Search strategy

Pubmed	(((((white spot[Title/Abstract]) OR (decalcification[Title/Abstract])) OR (remineralization[Title/Abstract])) OR (demineralization[Title/Abstract])) OR (caries[Title/Abstract])) AND ((randomized clinical trial[Title/Abstract]) OR (randomized controlled trial[Title/Abstract])) Filters: from 2015 – 2020
Scopus	((TITLE-ABS-KEY (white AND spot) OR TITLE-ABS-KEY (caries) OR TITLE-ABS-KEY (demineralization) OR TITLE-ABS-KEY (remineralization) OR TITLE-ABS-KEY (decalcification))) AND ((TITLE-ABS-KEY (randomized AND clinical AND trial) OR TITLE-ABS-KEY (randomized AND controlled AND trial))) AND (LIMIT-TO (PUBYEAR , 2021) OR LIMIT-TO (PUBYEAR , 2020) OR LIMIT-TO (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015)) AND (LIMIT-TO (SUBJAREA , "DENT"))
Embase	'white spot' OR 'caries'/exp OR caries OR 'decalcification'/exp OR decalcification OR 'demineralization'/exp OR demineralization OR 'remineralization'/exp OR remineralization AND 'randomized clinical trial' OR 'randomized controlled trial'/exp OR 'randomized controlled trial' OR rct AND (2015:py OR 2016:py OR 2017:py OR 2018:py OR 2019:py OR 2020:py OR 2021:py) AND ('adverse device effect'/dm OR 'adverse event'/dm OR 'demineralization'/dm OR 'dental caries'/dm OR 'dentin sensitivity'/dm OR 'gingiva bleeding'/dm OR 'gingiva disease'/dm OR 'gingivitis'/dm OR 'malocclusion'/dm OR 'periodontal disease'/dm OR 'periodontitis'/dm OR 'pulpitis'/dm OR 'tooth disease'/dm OR 'tooth pain'/dm OR 'tooth plaque'/dm OR 'tooth pulp disease'/dm) AND 'randomized controlled trial'/de

Assessment of the risk of bias in the included studies

Two review authors independently assessed the risk of bias for the included trials using Cochrane's risk of bias tool.[15] The study was judged as low risk if bias of all the domains were low. If at least one domain was considered high risk, the study was judged as high risk. A moderate risk of bias was considered if at least one domain was judged as unclear.

Results

Twenty-three papers met the qualifications for this systematic review. The literature involved both prevention and treatment of WSLs during and after orthodontic treatment.

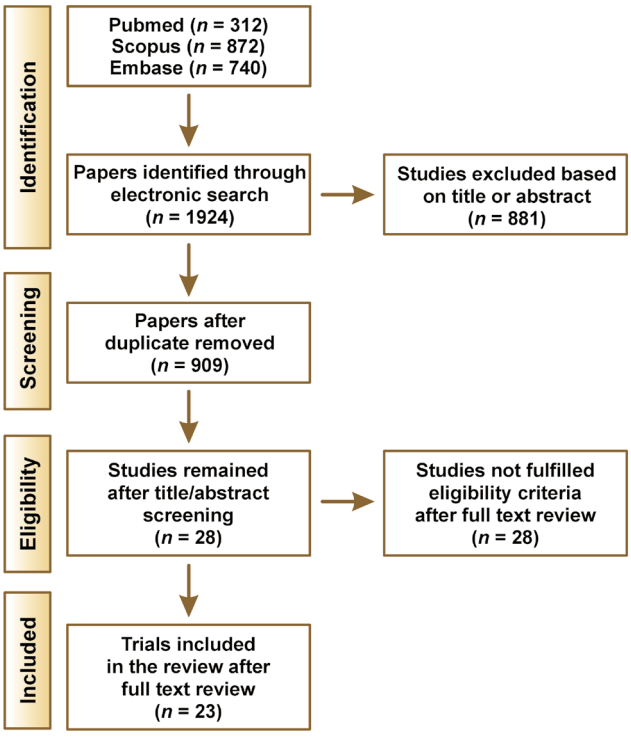

Fig. 1. Flow diagram of the study

Eleven papers addressed preventive strategies. One study was on bonding agents (fluoride-containing adhesive resin),[16] six on fluoride/ CPP-ACP containing products (varnish, toothpaste, mouth rinse, MI Paste Plus , etc.),[17–23] one study on photodynamic therapy,[24] one on special sealant,[25] and two studies were on CO2 laser.[26,27]

Twelve papers considered therapeutic strategies. Of these, seven studies were on remineralizing agents,[28–34] two studies were on resin infiltration,[35,36] one study evaluated the effectiveness of resin infiltration and microabrasion,[37] one study compared fluoride varnish and chlorhexidine mouthwash, and one study compared resin infiltration with varnish.[38] Table 3 demonstrates the general characteristics of the twenty-three included papers.

Risk of bias assessment

The details of the quality assessment of twenty-three eligible studies included in this systematic review were extracted and assessment of the risk of bias for included studies was performed. Two studies were assessed to be low, two studies were considered to be moderate, and nineteen studies were classified as high risk of bias (Table 4).

Discussion

WSL development is a common adverse effect following fixed orthodontic treatment which compromises the esthetic outcomes of treatment. Therefore, prevention and management of WSLs is extremely important for clinicians. In this review, we assessed recently published randomized clinical trials concerning both prevention and treatment of post-orthodontic WSLs.

Despite the breadth of literature concerning this issue, there is still no consensus on how to prevent or treat post-orthodontic WSLs. However, it is always easier and more effective to prevent rather than treat.

Table 3. Summary of the included studies

No.	Study	Number of participants	Age	Areas studied/ location of lesions	Caries risk assessment	Type of intervention	Duration	Diagnostic method	Conclusions
Prevention									
1	Kumar et al 2015	40	12–20 years	incisors and canines	-	RMGI varnish (split mouth)	6 months	DIAGNOdent direct visual inspection	resin-modified glass ionomer cement varnish had an effective role in prevention of WSLs during orthodontic treatment
2	Kirschneck et al 2016	90	10–17 years	all maxillary and mandibular dental surfaces with orthodontic brackets	ICDAS index of <2	placebo varnish (30) elmex® (30) (10000ppm) Fluor Protector S (30) (7700ppm)	4 weeks 12 weeks 20 weeks	ICDAS	Not any significant difference in the mean ICDAS in 3 groups / ICDAS index increased in 3 groups in T3
3	Hammad et al 2016	50	12–18 years	all maxillary and mandibular dental surfaces with orthodontic brackets	adequate oral hygiene	SeLECT DefenseTM sealant (25) Control (25)	12 months	Plaque Index (API)	sealant showed no significant effect as a solo preventive strategy.
4	Perrini et al 2016	24	mean age of 14 years 1 month.	Anterior, Middle, Posterior teeth	-	5% Varnish every 3 months (13) Varnish applied every 6 months (11) Controls (Split mouth)	3 months 6 months 9 months 12 months	DIAGNOdent	Periodic application of varnish can offer some protection against WSLs, but not to a statistically significant degree if the patients have excellent oral hygiene
5	Alabdullah et al 2017	34	13–25 years	all maxillary and mandibular dental surfaces with orthodontic brackets	adequate oral hygiene	fluoride-containing adhesive resin control (split mouth)	3 months 6 months 9 months 12 months	photograph Visual-inspection DIAGNOdent	Fluoride-containing resin adhesive does not have the desired preventive effect to prevent demineralization and WSL formation
6	Gomez et al 2018	20	12-18 years	Tooth surfaces with an orthodontic bracket	ICDAS index <1	Photodynamic Therapy (10) ultrasonic scaler (10)	3 months 6 months 9 months	ICDAS	No significant difference in the mean ICDAS Index between two groups / index slightly increased from T2 follow-up in both groups
7	Rechmann et al 2018	40 (37 for follow up)	11 years and Older	facial surfaces of incisors, canines, and first bicuspids	EDI mean: control 37.7 Experimental 42.9 ICDAS mean: Control 21.1 Experimental 21.9	Toothpaste + varnish (every 3 months) + MI paste plus (19) Toothpaste + mouthrinse (control) (18)	3 months 6 months 12 months	EDI ICDAS	no statistically significant differences in EDI sum and ICDAS scores between 2 groups
8	Raghis et al 2018	26	14 and 25 years	-	good oral hygiene	10.6 µm CO2 laser irradiation after bonding Control non-therapeutic light (split mouth)	1 month 2 month 6 month	clinical and photographic examinations digital images DIAGNOdent	10.6 µm CO2 laser has an inhibitory effect on demineralized lesions formation during orthodontic treatments.
9	Kau et al 2019	120 (100 for follow up)	12 years and Older	Facial surfaces of up to 20 teeth evaluated for enamel decalcification.	EDI mean: Clinpro 5000: 100 Clinpro Cr`eme: 97.7 MI Paste: 99.8	Clinpro 5000 (35) Clinpro Tooth Crème (32) MI paste plus (33)	1 month 2 months 3 months 4 months	Enamel Decalcification Index (EDI)	All treatments have a reduction effect on white spot lesions/ Clinpro 5000 showing a marginally better effect than the two other
10	Mahmoudzadeh et al 2019	95	12–30 years	Maxillary anterior teeth	-	0.4 mw, 10.6 µm, 5 Hz for 20 s, CO2 laser after bonding (278) placebo light control (276)	6 months	Visual inspection (WSLs' incidence) EDI (WSLs' extent) Clinical assessment (WSLs' severity)	CO2 laser effectively prevent incidence of WSLs, its effectiveness varied depending on the surface region.
11	Sonesson et al2020	182 (148 completed the trial)	12–18 years	bonded maxillary teeth	-	Varnish Ammonium fluoride 1.5% (75) every 6 week Placebo varnish (73)	Until the end of treatment	Digital photos	Ammonium fluoride reduces the prevalence of severe lesions (score 3 and 4).

No.	Study	Number of participants	Age	Areas studied/ location of lesions	Caries risk assessment	Type of intervention	Duration	Diagnostic method	Conclusions
Treatment									
12	Eckstein et al2015	20 (9 for follow up)	13-19 years	Quadrants with noncavitated, postorthodontic WSLs		Resin infiltration (split-mouth)	6 months 12 months	Spectrophotometer	esthetic improvement of postorthodontic WSL with resin infiltration
13	Singh et al 2016	41	16-25 years	labial surface of each tooth in both the jaws from central incisor to the first molar on either side		Control/ Fluoride Toothpaste (14) Toothpaste + varnish5 % (13) Toothpaste + CPP-ACP plus(14)	1 month 3 months 6 months	DIAGNOdent	Twice daily application of fluoride varnish or CPP-ACP plus crème along with fluoride toothpaste for 6 months has significant effect on remineralization of WSLs and decreased the severity of WSLs No additional benefit from using fluoride varnish and CPP-ACP
14	Restrepo et al 2016	35	13-20 years	Facial surfaces of anterior teeth and premolars		5% NaF varnish (12 patients, 2 apps one-week interval) 2% chlorhexidine gel (12 patients, 2 apps one-week interval) home care control(11 patients)	1 week 1month 2 months 3 months	DIAGNOdent Nyvad criteria	F and CHX were capable of controlling the WSLs adjacent to bracket/ F induced faster remineralization than CHX
15	He et al 2016	240 (211 for follow up)	12-25 years	All maxillary anterior teeth with a WSL but no cavitary caries		Fluoride varnish 5%(69) Fluoride film (Sheer) 5% (70) control (72)	3 months 6 months	Quantitative light-induced fluorescence	Treatment with either fluoride varnish or film can induce the greatest amount of remineralization in comparison with control group. Fluoride varnish may be slightly more effective than fluoride film.
16	Karabekiroglu et al 2017	41 (34 for follow up)	14-20	buccal surfaces of the anterior teeth, premolars, and first molars in the upper and lower jaws		CPP-ACP (16) Control (18)	36 months	DIAGNOdent Gorelick index ICDAS II criteria	Daily application of ACP-CPP was not significantly effective
17	Bock et al 2017	46 (39 for follow up)	>11 years	Four upper front teeth		1.25 per cent fluoride gel (21) Control (18)	1 week 2 weeks 6 weeks 12 weeks 24 weeks	digital intraoral photographs (WSL dimension) (WSL luminance)	no significant positive effect of high-dose fluoride on post-orthodontic WSL development (based on a photographical assessment of WSL luminance) Dimensional WSL quantification showed limited reliability
18	Bock et al 2017	46 (39 for follow up)	>11years	Four upper front teeth		1.25 per cent fluoride gel (21) Control (18)	1 week 2 weeks 6 weeks 12 weeks 24 weeks	Modified WSL index Lesion activity assessment (LAA)	No significant positive effect of high-dose fluoride (1.25 percent) on clinical post-orthodontic WSL was seen when compared with the placebo
19	Heravi et al 2018	36	13-23 years	Labial surfaces of six maxillary anterior teeth		MI paste plus (CPP-ACP and F) (12) Remin Pro (Hydroxyapatite and F) (12) Control (12)	4 weeks 8 weeks 12 weeks	Fluorescent camera	Both treatments are effective in reducing the area, increasing the mineral content, improving the appearance
20	Beerens et al 2018	51	12-19 years	Buccal surfaces of maxillary and mandibular teeth		MI Paste Plus (25) Placebo (26)	6 weeks 3 months 6 months 12 months	quantitative light-induced fluorescence (QLF) lesion changes scored visually	a significant improvement in enamel lesions over time in both groups with no differences between groups
21	Kannan et al 2019	12	14-30 years	Teeth with WSL after removal of fixed orthodontic appliances		Icon® resin infiltration (6) Clinpro™ XT varnish (6)	immediately 3 months 6 months	Spectrophotometer DIAGNOdent	Immediately after the intervention, Icon® resin infiltration demonstrated a significantly better improvement than Clinpro™ XT varnish. Conversely, at 3 and 6 months, Clinpro™ XT varnish showed significantly better improvement.
22	Knosel et al 2019	20 (8 for follow up)	12-17 years	Nonrestored, noncavitated postorthodontic WSL after multibracket treatment		Resin infiltration (split-mouth)	6 months 12 months +24 months	Spectrophotometer	Suitable for long term esthetic appearance improvement
23	Gu et al 2019	20 (16 available at T 12)	12-19 years	Anterior maxillary or mandibular teeth with WSLs		Resin infiltration (54) Microabrasion (54) Control (108) (split-mouth)	1 week 6 months 12 months	Photographs Spectrophotometer	Improved appearance of WSL with resin infiltration and microabrasion/ higher effect of resin infiltration at 12months

Table 4. Summary of the risk of bias for RCT studies according to thethe Cochrane Collaboration tool for assessing risk of bias

No.	Study	Design	Random sequence generation	Allocation concealment	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Risk of bias
1	Heravi et al	RCT	Low	Low	Low	Low	Unclear	Moderate
2	Karabekiroglu et al	RCT	Low	High	Low	High	Unclear	High
3	Singh et al	RCT	Low	Low	Low	High	Low	High
4	Gu et al	RCT	Low	Low	Low	High	Low	High
5	Knosel et al	RCT	Low	High	High	High	Low	High
6	Eckstein et al	RCT	Low	High	High	High	Low	High
7	He et al	RCT	Low	Low	Low	High	Low	High
8	Bock et al	RCT	Low	Low	Low	High	Low	High
9	Beerens et al	RCT	Low	Low	Low	High	High	High
10	Kannan et al	RCT	Low	High	Unclear	High	Low	High
11	Bock et al	RCT	Low	Low	Low	High	Low	High
12	Restrepo et al	RCT	Low	Low	Low	High	High	High
13	Kumar et al	RCT	Low	Unclear	Low	High	High	High
14	Kirschneck et al	RCT	Low	Low	Low	Low	Low	Low
15	Perrini et al	RCT	Low	High	Unclear	Low	Low	High
16	Gomez et al	RCT	Low	Low	Low	High	High	High
17	Kau et al	RCT	Low	Low	Unclear	High	High	High
18	Hammad et al	RCT	Low	Low	Low	High	Unclear	High
19	Rechman et al	RCT	Low	Low	Low	High	Unclear	High
20	Alabdullah et al	RCT	Low	Low	Low	High	Low	High
21	Raghis et al	RCT	Low	Low	Low	Low	Low	Low
22	Mahmoudzadeh et al	RCT	Low	Low	Low	High	Unclear	High
23	Sonesson et al	RCT	Low	Low	Low	Low	Unclear	Moderate

The current literature contains invaluable reviews concerning white spot lesions.[39–43] However, some of these have focused on a single remineralizing agent,[39,42,43] and the others were limited to assessing remineralizing agents without addressing contemporary approaches such as laser irradiation,[40,41,43] and some were not focused on orthodontically induced lesions.[39,40] Another advantage of the current study was the evaluation of both preventive and treatment approaches together. In addition, the current study summarized further information such as location of the lesions and age group of the participants (Table 3). This shows that most of the included studies evaluated the labial surfaces of the teeth with brackets, which is sensible due to the prevalence and mechanism of white spot lesions. However, the studies were limited to patients under 30 years old, which reveals the need for studies on prevention and treatment of lesions in older patients. In the current study, we tried to overcome the shortages with a more comprehensive review.

The diversity in the results from clinical trials can be due to variable methods and study designs, a wide range of products claiming effectiveness on WSL treatment, various methods for the detection and quantification of WSLs, and different procedures for the application of active agents. Visual detection indices including International Caries Detection and Assessment System (ICDAS) and EDI, spectrophotometer, DIAGNOdent, and QLF are the most common methods used for detection and quantification of WSLs. Using the naked eye is not a reliable indicator, whereas magnifying loupes accompanying air drying of tooth surface is an effective method for identifying WSLs of smooth surfaces. However, there is no significant difference between DIAGNOdent and loupes.[44] Since all studies using visual indices for detecting or assessing improvement of WSLs has depended on the naked eye only and did not incorporate magnifying loupes, minor improvement of lesions might have been ignored which can directly influence the studies' results.

Prevention

Results of this review emphasize the pivotal role of routine oral hygiene maintenance in prevention of WSLs. Regular brushing with two different fluoride dentifrices (Clinpro 5000 and Clinpro Tooth Creme) twice daily for two minutes was effective in hindering WSLs, with results comparable to those of MI Paste Plus.[20] The importance of routine oral hygiene procedures is also noted by Rechmann et al. who demonstrated that the combination of fluoride tooth paste and fluoride varnish and MI Paste

Plus together does not provide any preventive advantage over the routine use of fluoride tooth paste and mouth rinse. In addition, it has been shown that using 5% sodium fluoride varnish is effective against WSL formation only in patients with suboptimal oral hygiene, and it is not useful in those with perfect oral hygiene.[19] Similarly, a single dose of elmex® fluid (10,000 ppm) and Fluor Protector S (7700 ppm) had no benefit over the routine sufficient dental hygiene approach, and application of 1.5% ammonium fluoride every six weeks only reduced the number of severe lesions (which are more likely to be sighted in patients with poor oral hygiene).[13,17]

Several interventions such as photodynamic therapy, using ultrasonic scaler and the application of sealants have been tested for their capability against WSLs. However, none of them were successful in the prevention of WSLs.[24,25] Resin Modified Glass Ionomer cement (RMGIC) has shown promising results in preventing WSL development during orthodontic treatment.[22] However, one split mouth RCT revealed no preventive effect of fluoride-releasing resin composite against demineralization and development of WSLs adjacent to brackets.[16]

There is ample evidence that laser irradiation makes enamel more resistant to acid-induced demineralization by altering its structure.[45] Various types of lasers have been introduced for this purpose, including CO2, erbium-doped yttrium aluminum garnet (Er: YAG), neodymium-doped yttrium aluminum garnet (Nd: YAG), erbium, chromium: yttrium-scandium-gallium-garnet (Er, Cr: YSGG), diode, and argon lasers. However, studies that assessed laser effectiveness in reducing enamel susceptibility were mainly in vitro and were not included in this review.[46] There is supporting data for effectiveness of CO2 laser in the prevention of WSL formation. In 2019, Mahmoudzadeh et al. exposed teeth to CO2 laser (0.4 mw, 10.6 μm, 5 Hz) for 20 s following bracket attachment and uncovered that CO2 laser irradiation not only caused a reduction in WSL incidence, but it also diminished their extent and severity after 6 months.[26] Raghis et al. also concluded that CO2 laser irradiation had an inhibitory effect on WSL formation during orthodontic treatment after 2 and 6 months.[27] The effectiveness of laser irradiation in preventing enamel demineralization was evaluated in a systematic review in 2018; however, more RCTs are needed to verify the clinical efficacy of different available systems.[45] In addition, the cost-effectiveness of using lasers should be assessed in comparison to the more economical and accessible traditional approaches, such as using fluoride varnish.

Treatment

Careful supervision and waiting for at least 3-6 months until natural remineralization occurs is the method of choice in most patients. The optimal use of fluoride-containing toothpastes, which is twice daily without excessive rinsing after brushing, must be carefully monitored during this period.[13,47,48] However, natural remineralization is insufficient in many cases, and sometimes it doesn't take place at all. Therefore, in many patients, adjunctive considerations should be considered.

Results of this review revealed that MI Paste Plus does not provide any long term benefits in the treatment of WSLs, while short term application of it has shown controversial results.[28,34] All reviewed clinical trials strongly support using 5% sodium fluoride varnish as an effective treatment for WSLs.[29,30,35–38]

In 2018, Fernandez et.al concluded that most remineralizing agents were not more effective than conventional oral hygiene protocols, and the only effective product for remineralization of WSLs was 5% sodium fluoride varnish applied professionally once per month for 6 months.[49] Although application of fluoride varnish on a monthly basis can effectively lead to reversal of WSLs after debonding, the use of high concentration fluoride immediately after debonding is under question, perhaps because surface hyper-mineralization may arrest remineralization and increase the risk of permanent brown discoloration.[2,50]

Despite the positive results of fluoride varnish, the effectiveness of using 1.25% fluoride gel for treatment of WSLs is not supported by the literature.[32,33] Application of ACP-CPP alone has not shown significant improvement of WSLs, although it is effective when combined with daily use of fluoride dentifrices.[29,31]

Similar to fluoride varnish, resin infiltration has also shown successful results for the treatment of WSLs. Comparison of these two methods demonstrated that resin infiltration shows greater improvement immediately after intervention, while fluoride varnish displays significantly better results over time.[38] One split mouth study that compared resin infiltration with microabrasion illustrated esthetic improvement of WSLs with both techniques, but resin infiltration showed better enhancement at 12 months.[37]

None of the included studies compared microabrasion with a control group. Only one paper made a comparison between resin infiltration and microabrasion as discussed above.[37] Microabrasion is an invasive method which is capable of masking more severe and long-standing lesions; however, care must be taken, as this is a sensitive method and should be repeated several times.[51] No recent studies concerning bleaching of post-orthodontic WSLs fulfilled the inclusion criteria.

Conclusions

Observation of oral hygiene by brushing with fluoride toothpaste is the backbone of prevention of WSLs in patients undergoing orthodontic treatment. However, application of 5% fluoride varnish or using a CO2 laser after bonding are recommended in patients with compromised oral hygiene. If WSLs are formed during fixed orthodontic treatment, fluoride varnish 5% and resin infiltration are effective methods for treatment.

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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