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Knowledge and awareness of dental specialists, general dentists and dental assistants regarding SARS-CoV-2

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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. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has had drastic effects among healthcare professionals.

Objectives. This study aimed to assess knowledge and awareness among dental specialists, general dentists and dental assistants regarding standard patient care guidelines for minimizing the spread of the infection.

Material and methods. A survey-based cross-sectional study was conducted on a sample of 84 participants. The participants were divided into groups of dental specialists, general dentists and dental assistants. A modified version of a validated questionnaire was used to evaluate knowledge and awareness regarding SARS-CoV-2 as per the Centers for Disease Control and Prevention (CDC) guidelines. Data was analyzed using the one-way analysis of variance (ANOVA) and post-hoc Tukey's tests to assess differences in knowledge regarding the SARS-CoV-2 guidelines across the 3 groups. The simple linear regression analysis was used to examine factors influencing the knowledge scores.

Results. Among all dental specialists, 13 orthodontists, 9 operative dentists, 8 maxillofacial surgeons, 4 prosthodontists, and 1 periodontist responded to the survey. The mean knowledge scores of dental specialists, general dentists and dental assistants were 10.05 ± 2.10 , 9.95 ± 2.30 and 8.53 ± 2.10 , respectively. Overall, we found a significant difference ($p = 0.02$) in the knowledge scores between the groups, and pairwise comparisons showed that there was a significant difference ($p = 0.03$) in the knowledge scores between dental specialists and dental assistants.

Conclusions. These findings suggest that hospitals should conduct mandatory workshops, training sessions and seminars to raise the awareness of the novel coronavirus pandemic and disinfection protocols, not only for specialists, but for all staff members.

Keywords: cross-infection, health knowledge, attitudes, practice

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Introduction

A wave of respiratory tract infections first occurred in Wuhan, China, in December 2019 and quickly became the leading challenge for public health globally.¹ Due to similarities in the RNA genome to the virus found in the respiratory tract of individuals infected with severe acute respiratory syndrome coronavirus (SARS-CoV), the World Health Organization (WHO) named the novel coronavirus responsible for these infections SARS-CoV-2.²

The symptoms of the SARS-CoV-2 infection vary from person to person, and may range from mild fever and a dry cough to severe acute respiratory distress and cardiac arrhythmias due to low oxygen saturation.^{3,4} Studies⁴⁻⁶ have found that this virus is transmitted in the form of droplets via coughing, sneezing or talking with an infected person at a minimal distance.^{5,6} For this reason, SARS-CoV-2 is spreading all over the globe irrespective of socioeconomic status, race or continent.^{7,8} Due to the high mortality rate and rapid spread of the infection, healthcare professionals declared an emergency at hospitals to ensure patient care.⁹⁻¹⁰ The WHO declared dental surgeons to be at the highest risk of infection among healthcare professionals, as SARS-CoV-2 is transmitted via oral and nasal secretions in the form of droplets.¹¹ In addition, the probability of transmission is increased in this setting, as dental procedures can generate aerosol while using instrumentation in the oropharyngeal region.¹²⁻¹⁴

Due to the fear of SARS-CoV-2 transmission and lockdowns, private and public dental practices faced drastic consequences. However, due to unavoidable maxillofacial emergencies, dental clinics could not shut down completely.¹⁵ Therefore, dental surgeons had a huge responsibility to ensure patient safety and care while conducting emergency procedures. Patient safety can only be assured if dental surgeons and dental staff members are properly prepared and well aware of the COVID-19 pandemic situation.¹⁶

Researchers have observed that the signs and symptoms, mode and rate of transmission, survival, and rate of recovery/mortality of infected individuals vary geographically.¹⁰⁻¹⁴ However, the WHO provided standard patient care guidelines for healthcare setups all over the world.¹⁷ All dental professionals must be well aware of standard infection control protocols to ensure patient safety and care.^{18,19} This study aimed to assess knowledge and awareness among dental specialists, general dentists and dental assistants regarding the general features of SARS-CoV-2. The secondary objective of this study was to identify factors influencing the level of knowledge and awareness of dental healthcare professionals regarding standard patient care guidelines in controlling the spread of infection. Thus, the null hypothesis of this study was that there is no difference in the knowledge and awareness scores among dental healthcare professionals regarding patient care protocols during the SARS-CoV-2 pandemic.

Material and methods

A survey-based cross-sectional study was conducted in May–June 2020 after ethical approval was obtained from the institutional ethics review committee (No. 2020-5281-11488). A required sample size of 72 participants was calculated using one-sample mean online software (https://www2.ccrb.cuhk.edu.hk/stat/mean/osm_equivalence.htm), selecting a variance of 25, based on an error of estimate of 5, an equivalence limit of 2, a confidence level of 95%, and a power of 80%. Considering the chances of dropouts, the sample size was inflated by 10%, for a total of 84 participants. The participants were divided into 3 groups: dental specialists; general dentists; and dental assistants.

Data was collected using a modified version of a validated questionnaire designed by Khader et al.²⁰ (Table 1) to evaluate the awareness of dental specialists, general dentists and dental assistants regarding SARS-CoV-2 as per the Centers for Disease Control and Prevention (CDC) guidelines. This questionnaire had 2 parts. The 1st part included information on demographics, such as sex, age, workplace, and experience, and the 2nd part consisted of questions regarding the knowledge and awareness of SARS-CoV-2. Among all the options, only one answer was considered to be correct. The maximum score any participant could secure was 15.

The survey was conducted via Google forms and participants were recruited using social media platforms. All participants were asked to fill out the hard and soft copies of the forms. Five reminders were sent to the non-responders before exclusion. The recruitment of participants was done based on the following inclusion criteria: dental specialists with a minimum of 2 years of experience in any specialty; practicing general dentists with 16 years of education; and practicing dental assistants with a minimum of 12 years of education.

Statistical analysis

Data was analyzed using the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA), and Software for Statistics and Data Science, v. 12.0 (StataCorp, College Station, USA). The Shapiro–Wilk test showed a normal distribution of the data. The descriptive analyses of the variables were reported using means (*M*) and standard deviations (*SD*). The one-way analysis of variance (ANOVA) was applied to assess differences in the scores of knowledge and awareness regarding SARS-CoV-2 guidelines across the 3 groups of dental healthcare professionals. Pairwise comparison were also performed using post-hoc Tukey's tests. In addition, the univariate and multivariate linear regression analyses were used to examine the influence of the variables on the knowledge and awareness scores regarding the SARS-CoV-2 pandemic.

Table 1. Percentage [%] of correct answers among the 3 groups of dental healthcare professionals

Knowledge and awareness regarding novel coronavirus disease 2019 (COVID-19) questionnaire		Dental healthcare professionals		
		dental specialists	general dentists	dental assistants
Q.1	Where did the disease outbreak start?	97.1	79.2	96.0
Q.2	What is the name of the virus causing SARS-CoV-2 infection?	45.7	41.7	40.0
Q.3	What is the mode of transmission of this disease?	31.0	50.0	48.0
Q.4	Which age group is most susceptible to the disease?	40.0	66.7	32.0
Q.5	After how many days may an infected person show signs and symptoms?	85.0	54.2	52.0
Q.6	What are the signs and symptoms of mild SARS-CoV-2 infection?	91.4	100.0	84.0
Q.7	If a person had contact with an infected person, what should he/she do?	71.4	83.3	52.0
Q.8	What are the chances of recovery after getting infected with SARS-CoV-2?	74.3	79.8	60.0
Q.9	What should a person with a recent travel history and symptoms do?	85.7	66.7	20.0
Q.10	What is the incubation period of SARS-CoV-2?	42.9	33.3	60.0
Q.11	What are the possible complications of the SARS-CoV-2 infection?	85.7	95.8	56.0
Q.12	Which profession is considered at the highest risk of being infected with SARS-CoV-2 by the WHO?	60.0	50.0	72.0
Q.13	What is the PPE protocol for dentists while treating an asymptomatic patient with a history of exposure to a SARS-CoV-2- positive patient?	97.1	70.8	96.0
Q.14	Which of the following hand hygiene techniques can prevent the transmission of infection among dentists?	80.0	37.5	48.0
Q.15	What is the preferred method of hand hygiene for healthcare professionals?	17.1	37.5	32.0

PPE – personal protective equipment.

Results

Demographics

A summary of the descriptive analysis of all variables among the 3 groups is shown in Table 2. The sample included 45 males and 39 females. The comparison of scores across sex found a significant difference ($p = 0.03$) in knowledge in the univariate analysis.

The mean age of the total sample was 35.17 ± 9.5 years. A comparable mean age was observed across the groups of dental specialists, general dentists and dental assistants. Among dental specialists, 13 were orthodontists, 9 were operative dentists, 8 were maxillofacial surgeons, 4 were prosthodontists, and 1 was a periodontist.

Table 2. Descriptive statistics for the participants

Profession	Sex <i>n</i> (%)	Age [years] <i>M</i> \pm <i>SD</i>	Experience [years] <i>M</i> \pm <i>SD</i>	Workplace <i>n</i>	Knowledge scores <i>M</i> \pm <i>SD</i>
Dental assistants	M = 17 (68.0) F = 8 (32.0)	34.6 \pm 11.4	8.8 \pm 6.8	PuH = 19 PH = 4 PC = 2	8.7 \pm 2.1
General dentists	M = 13 (54.2) F = 11 (45.8)	31.5 \pm 5.8	8.7 \pm 7.4	PuH = 14 PH = 5 PC = 5	9.9 \pm 2.6
Orthodontists	M = 5 (38.5) F = 8 (61.5%)	35.6 \pm 4.8	10.0 \pm 5.2	PuH = 2 PH = 10 PC = 1	10.4 \pm 1.6
Operative dentists	M = 2 (22.2) F = 7 (77.8)	30.5 \pm 7.0	8.2 \pm 5.3	PuH = 2 PH = 7	10.0 \pm 1.2
Maxillofacial surgeons	M = 3 (37.5) F = 5 (62.5)	45.8 \pm 9.5	23.6 \pm 12.5	PuH = 2 PH = 6	89.2 \pm 3.7
Prosthodontists	M = 4 (100)	47.2 \pm 5.9	19.0 \pm 1.1	PH = 4	10.2 \pm 1.2
Periodontists	M = 1 (100)	36.0 \pm 0.0	4.0 \pm 0.0	PH = 1	12.0 \pm 1.1

N = 84; *M* – male; *F* – female; *M* – mean; *SD* – standard deviation; PuH – public hospital; PH – private hospital; PC – private clinic.

Knowledge scores regarding SARS-CoV-2

The mean knowledge score for dental specialists was 10.05 ± 2.10 , for general dentists – 9.95 ± 2.30 and for dental assistants – 8.53 ± 2.10 (Table 3). There was an overall significant difference ($p = 0.02$) in the knowledge and awareness scores across these groups of dental professionals. Pairwise comparisons revealed a significant difference ($p = 0.03$) in the knowledge and awareness scores between dental specialists and dental assistants (Table 4).

Table 3. Comparison of the knowledge scores across the groups of dental healthcare professionals (one-way ANOVA)

Profession	Knowledge scores	<i>p</i> -value
Dental specialists <i>n</i> = 35	10.05 ±2.10	0.02*
General dentists <i>n</i> = 24	9.95 ±2.30	
Dental assistants <i>n</i> = 25	8.53 ±2.10	

N = 84; * statistically significant ($p \leq 0.05$).

Among all dental specialists, we found that the periodontist and orthodontists were well aware of the general CDC guidelines, with the mean scores of 12.0 ± 1.1 and 10.4 ± 1.6 , respectively. The mean scores of operative dentists (10.0 ± 1.2) were comparable with those of orthodontists (Table 2). The regression model showed that there were no significant differences in scores between the specialties (Table 5 and Table 6).

We also found that almost 97% of dental healthcare professionals responded accurately with regard to the origin of SARS-CoV-2 infection and its initial symptoms.

Table 4. Pairwise comparisons among dental healthcare professionals (Tukey's post hoc test)

Comparison	<i>p</i> -value
Dental specialists vs dental assistants	0.03*
Dental specialists vs general dentists	0.98
General dentists vs dental assistants	0.07

N = 84; * statistically significant ($p \leq 0.05$).

Table 5. Factors influencing the knowledge scores among dental healthcare professionals (simple linear regression model)

Variables	Beta coefficient	95% CI	<i>p</i> -value	<i>R</i> ²
Male sex	-0.02	-1.04, 1.00	0.96	0.001
Age [years] (20–30 as reference)	31–40	0.59	-4.38, 1.66	0.25
	41–50	-0.21	-1.58, 1.15	0.75
	51–60	2.90	0.90, 4.91	≤0.001**
	>60	-8.29	-12.50, -4.07	≤0.001**
Profession (general dentists as reference)	dental assistants	-1.43	-2.75, -0.12	0.03*
	orthodontists	0.50	-1.07, 2.08	0.52
	operative dentists	0.04	-1.75, 1.83	0.96
	maxillofacial surgeons	0.70	-2.58, 1.16	0.45
	prosthodontists	0.04	-2.43, 2.52	0.97
Workplace (private hospitals as reference)	periodontist	2.04	-2.64, 6.73	0.38
	public hospitals	1.31	-0.63, 2.89	0.20
private clinics	-1.58	-1.51, 1.19	0.81	0.040

N = 84; CI – confidence interval; * statistically significant ($p \leq 0.05$); ** statistically significant ($p \leq 0.001$).

Table 6. Factors influencing the knowledge scores among dental healthcare professionals (multivariable linear regression model)

Variables	Beta coefficient	95% CI	<i>p</i> -value	<i>R</i> ²
Age [years] (20–30 as reference)	31–40	0.34	-0.77, 1.46	0.53
	41–50	-1.47	-3.19, 0.25	0.09
	51–60	2.25	0.24, 4.25	0.02*
	>60	-9.84	-14.24, -5.44	≤0.001**
Profession (general dentists as reference)	dental assistants	-1.32	-2.75, -0.12	0.03*
	orthodontists	0.55	-1.07, 2.08	0.52
	operative dentists	0.12	-1.75, 1.83	0.96
	maxillofacial surgeons	1.12	-2.58, 1.16	0.45
	prosthodontists	1.92	-2.43, 2.52	0.97
periodontist	1.17	-2.64, 6.73	0.38	

N = 84; * statistically significant ($p \leq 0.05$); ** statistically significant ($p \leq 0.001$).

However, 68% of dental assistants, 60% of general dentists and 42% of dental specialists were not sure about the clinical protocols to minimize cross-infection.

Discussion

In the literature, there are several strategies outlined to deal with the SARS-CoV-2 infection.^{15–20} However, there is still no definitive treatment plan for this infection. The only possible way to deal with this healthcare crisis is to minimize the spread of SARS-CoV-2.¹⁴ Studies have found a strong association between the rapid spread of SARS-CoV-2 infection and the lack of knowledge regarding the infection, the personal protective equipment (PPE) and the guidelines for the PPE use among healthcare professionals.^{10,14,20}

Dental specialists, however, showed a greater awareness regarding the spread of the virus as compared to dental assistants and general practitioners. This verifies the perceived hypothesis that due to more interest in theoretical knowledge and frequent attendance at the infection control seminars arranged for specialists by the policy-making organizations, dental specialists are more aware. Hence, the null hypothesis was rejected.

Furthermore, healthcare professionals' knowledge scores indicated the interest of the participants to find a solution to this pandemic. However, we found that only 63% of dental healthcare professionals responded to the survey questions correctly. This can be attributed to the versatile nature of SARS-CoV-2 symptoms, the associated myths, confusion among policy makers, and the lack of awareness among dental healthcare professionals.

The lack of awareness regarding the transmission of SARS-CoV-2 among dental assistants is worrisome, and may lead to drastic consequences for dental healthcare professionals and the patients' safety. The findings of this study are in agreement with previous studies.^{20–22} Nemati et al. conducted a survey on Iranian nurses and found that 94.11% had a low level of knowledge regarding SARS-CoV-2.²² This can be attributed to the fact that the researchers conducted their study at the beginning of 2020, when all healthcare professionals were not well aware of the SARS-CoV-2 safety protocols and guidelines.

We also found that dental staff members in the private sector were better aware of SARS-CoV-2 as compared to those working in public hospitals. These results may indicate the lack of resources as well as seminars on infection control protocols, and an increased number of workers of a low socioeconomic status in the public sector.

Another finding was that only 27% of dental professionals were well aware of proper hand hygiene and disinfection techniques after dental procedures. However, Nemati et al. found this percentage to be 57%, which may have been due to the fact that the WHO had recently modified the CDC guidelines on infection control

at the time of that study.²² The results suggest that policy makers should take steps to disseminate the updated CDC guidelines and policies among healthcare professionals by arranging frequent meetings and seminars. Also, the WHO should arrange seminars, webinars and meetings with healthcare workers in developing countries to share the updated facts, procedures and protocols to control this pandemic.¹⁷


The findings of this study are specifically associated with the South Asian population. However, some studies have shown that variations in the features of transmission of this infection are based on geography and ethnicity.^{11–14} This indicates a need for the development of guidelines for all demographic variations. It should be noted that this research was performed on the basic general guideline questionnaire provided by the CDC to restrict the transmission of communicable diseases.

Conclusions

To increase knowledge for dealing with the COVID-19 pandemic, hospitals should conduct mandatory workshops and seminars for all specialists and staff. We can extract the following conclusions from this study: dental healthcare professionals are now more concerned about measures for preventing the spread of the infection; dental specialists are better informed about disinfection protocols and the control of cross-infection as compared to dental assistants; and dental healthcare professionals are at least aware of the guidelines regarding the use of PPE.

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Satisfaction and stress levels of dentistry students relating to distance education

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Abstract

Background. It is not known whether the coronavirus disease 2019 (COVID-19) pandemic has affected dentistry education.

Objectives. This study aimed to determine the satisfaction and stress levels of dentistry students in Turkey regarding distance education during the COVID-19 pandemic, and to evaluate their opinions on this matter.

Material and methods. This cross-sectional research study was conducted from October to November 2020 with the use of a web-based questionnaire consisting of 3 sections. The 1st section focused on demographic data. The 2nd section evaluated dentistry students' opinions regarding distance education during the pandemic; it comprised 8 multiple-choice questions and 1 open-ended question. The 3rd section referred to the 10-item Perceived Stress Scale (PSS-10), which is intended to assess the stress levels. The data was subjected to the descriptive statistical analysis, the χ^2 tests and the logistic regression analysis.

Results. The sample consisted of 919 dentistry students, reflecting a response rate of 84%. Of the total sample, 81.6% of the participants were studying at state universities and more than half were female. These students' perceived stress levels were significantly increased due to the following factors: education at a state school; insufficient technical opportunities at home/dormitory facilities; decreased support from the academic staff of the universities during distance education; and a low level of knowledge of online document usage ($p < 0.05$). The evaluation of the responses to the open-ended question indicated that the difficulties encountered by highly stressed students during distance education were mostly adaptation-related ($p = 0.011$). The logistic regression analysis showed that gender was significantly associated with high stress levels ($p < 0.05$).

Conclusions. The data showed that dentistry students preferred hybrid education and were unsatisfied with their experience with distance education.

Keywords: dental students, distance education, COVID-19, Perceived Stress Scale

Introduction

Coronavirus disease 2019 (COVID-19) is a new illness caused by severe acute respiratory syndrome coronavirus 2 (SARS CoV-2),¹ a member of the Coronaviridae family, that emerged in Wuhan, Hubei Province in China in December 2019.² COVID-19 has created a public health problem, affecting not only China, but the whole world. It spread widely to different countries, prompting the World Health Organization (WHO) to declare its occurrence a pandemic on March 11, 2020.³ The rapid spread of the virus, the ensuing threat to the entire world and its effects on the global economy have led to the implementation of different measures and practices, such as strict protective management and regulation through social distancing, flight bans and quarantine.

In dentistry, most treatment procedures involve the production of droplets and/or aerosols, which can cause infection; thus, the pandemic has also affected dentistry education. Governments and institutions have had to take swift and draconian measures for continuing education, and transition from face-to-face learning to distance learning.⁴ In distance education, students can benefit from remotely available educational opportunities, but there is a lack of face-to-face interactions, group work, classroom activities, responsibilities, and social development. In this type of educational delivery, students may encounter problems, such as the absence of timely and continuous support services, a lack of extracurricular social activities, insufficient communication infrastructure, and the inability to manage time effectively because of lecture intensity. Lecturer-induced problems can also arise, including the lecturer's inability to adapt to technology, a lack of preparation for student-centered content and the ineffective use of communication tools.⁵ Dentistry education involves pre-clinical and clinical practice as well as theoretical lectures. It is a hands-on type of learning involving teaching clinics, which requires a very high teacher-to-student ratio.⁶ Accordingly, the health and safety of patients, students and staff are very important. The COVID-19 pandemic has led to the reconsideration of many aspects of clinical training, including the organization of dental clinics, the control of aerosol release and airflow, the extension of clinical decontamination time, and the review of appropriate personal protective equipment (PPE).⁷ Shortcomings in these respects mean that some faculties are unable to provide sufficient amount of knowledge and skills to dentistry students within distance education.

In different countries, various studies have been conducted on the perceptions of university students, their experiences of significant changes in the educational system and the distance education process, and their satisfaction with this educational delivery method.^{5,8,9} These unexpected changes have evoked stress among students. Considering the effects of stress on human health, a necessary task is to measure and quantify this condition. One of the most frequently used measurement instruments for this

purpose is the Perceived Stress Scale (PSS)¹⁰ developed by Cohen et al.¹¹

Although studies on the stress levels and perceptions of dentistry students during the COVID-19 pandemic have been conducted, no such research has been carried out in Turkey. The present work was therefore initiated to determine the stress levels in dentistry students, and their satisfaction with theoretical and practical classes under distance education conditions during the pandemic.

Material and methods

This cross-sectional study was conducted from October to November 2020 with the use of a web-based survey. The research protocol was approved by the Ethics Committee of Mersin University, Turkey, and was executed in accordance with the most recent guidelines of the Declaration of Helsinki. The study was also performed following the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.¹² The sample size was calculated on the basis of the total number of dentistry students (21,305) enrolled at the dentistry faculties from 2016 to 2020 who were included in the distance education process as part of their institutions' response to the pandemic (www.openepi.com/SampleSize). The assumption of a 50% response rate and 95% confidence intervals (CI) yielded a minimum sample of 378 randomly selected individuals as a requirement.

An electronic questionnaire (Google Forms) was prepared by modifying the questionnaires previously validated by Rahali et al.,¹³ Asiry,⁹ and Halim and Sulaiman.¹⁴ A pilot test involving 30 students indicated no need for any adjustments to the questionnaire. Respondents for the web-based questionnaire were selected via probability sampling from a closed population list.¹⁵ The eligibility criteria were as follows: currently a dentistry student; over the age of 18; consented to participate in the study; and the completion of the questionnaire. Students under the age of 18 were excluded from this study (Fig. 1). A link to the questionnaire was sent to dentistry students at state

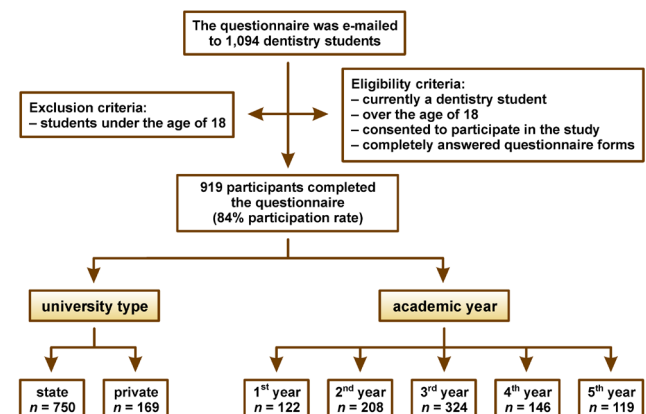


Fig. 1. Flowchart of the sample selection process

and private universities in Turkey via e-mail, an Internet communicator (WhatsApp) and social media. A reminder message was sent a week later to increase the participation rate and minimize the risk of bias.

The developed questionnaire consisted of 3 sections. Following the explanation of the purpose of the survey, the 1st section focused on the demographic data (gender, university type and academic year) of the participants (Table 1). The 2nd section was devoted to the students' opinions on distance education during the COVID-19 pandemic. It comprised 8 multiple-choice questions and 1 open-ended question. A Likert scale ranging from 1 to 5 was used as the response method for opinion-related questions (i.e., 1 – strongly disagree; 2 – disagree; 3 – uncertain; 4 – agree; and 5 – strongly agree). The major problem faced by the students during the distance education process was extracted through the open-ended question. The answers were categorized in terms of obstacles as follows: technical; financial; adaptation; practical training; pedagogical; and other (Table 1). The 3rd section of the questionnaire consisted of the 10-item PSS (PSS-10), which was designed to evaluate the stress levels of students as they deal with distance education. The PSS-10 referred to the students' thoughts and feelings over the previous month, using a 0–4 response scale.¹⁶ The PSS-10 covers 2 factors – the first one comprises 6 negative items measuring an individual's stress perception, whereas the other one comprises 4 positive items measuring coping or adaptation to stress.¹⁷ The scores assigned to questions 1, 2, 3, 6, 9, and 10 reflect a response scale of 0–4 (0 – never; 1 – hardly ever; 2 – sometimes; 3 – often; and 4 – very often) and are as follows: 0 – 0; 1 – 1; 2 – 2; 3 – 3; and 4 – 4. Answers to the remaining questions are reverse-scored. Individual scores on the PSS-10 can thus range from 0 to 40, with higher scores indicating greater perceived stress. Scores in the ranges of 0–13, 14–26 and 27–40 are considered indicative of low, moderate and high perceptions of stress, respectively (Table 1).

Table 1. Demographic data of the participants and questionnaire items related to distance education

Questionnaire items	n (%)
Gender	
– female	542 (59.0)
– male	377 (41.0)
University type	
– state	750 (81.6)
– private	169 (18.4)
Academic year	
– 1 st	122 (13.3)
– 2 nd	208 (22.6)
– 3 rd	324 (35.3)
– 4 th	146 (15.9)
– 5 th	119 (12.9)
Preferred device for participating in distance education	
– cellphone	272 (29.6)
– computer	183 (19.9)
– laptop	447 (48.6)
– tablet	17 (1.8)

Questionnaire items	n (%)
My opportunities (devices, access to the Internet, etc.) to participate in distance education classes from my home or dormitory are sufficient.	
– strongly disagree	70 (7.6)
– disagree	149 (16.2)
– uncertain	177 (19.3)
– agree	383 (41.7)
– strongly agree	140 (15.2)
On which platform or application do you follow your training?	
– Microsoft Teams	116 (12.6)
– Zoom	244 (26.6)
– Google Meet	102 (11.1)
– other	457 (49.7)
During the distance education process, lecturers responsible for classes supported you in solving your problems.	
– strongly disagree	52 (5.7)
– disagree	122 (13.3)
– uncertain	310 (33.7)
– agree	354 (38.5)
– strongly agree	81 (8.8)
I know how to open, modify and upload online documents.	
– strongly disagree	1 (3.4)
– disagree	80 (8.7)
– uncertain	178 (19.4)
– agree	475 (51.7)
– strongly agree	155 (16.9)
Which of the following best describes your preference for participating in classes?	
– traditional learning method (face-to-face)	303 (33.0)
– online education	145 (15.8)
– hybrid education	471 (51.3)
The distance education process is suitable for dentistry pre-clinical and clinical practice training.	
– strongly disagree	655 (71.3)
– disagree	149 (16.2)
– uncertain	59 (6.4)
– agree	23 (2.5)
– strongly agree	33 (3.6)
I am satisfied with distance education.	
– very satisfied	43 (4.7)
– satisfied	123 (13.4)
– neutral	218 (23.7)
– dissatisfied	247 (26.9)
– very dissatisfied	288 (31.3)
What is the major problem you encountered in the distance education process?	
– technical obstacles (problems with the access to the Internet)	145 (15.8)
– financial obstacles (not being able to secure a device)	41 (4.5)
– adaptation obstacles (difficulties with adapting to the situation)	106 (11.5)
– practical training obstacles (a lack of practical training)	292 (31.8)
– pedagogical obstacles (limited contact with faculty members)	219 (23.8)
– other	116 (12.6)
PSS-10 scores	
– low stress	84 (9.1)
– moderate stress	655 (71.3)
– high stress	180 (19.6)
PSS-10 total score	
Me (min–max)	21 (1–40)

PSS-10 – 10-item Perceived Stress Scale; Me – median; min – minimum; max – maximum.

Statistical analyses

Statistical analyses were performed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). Categorical measurements were summarized as numbers and percentages, whereas continuous measurements were expressed as median and minimum–maximum. The χ^2 tests were conducted to compare categorical variables. The logistic regression analysis was performed to determine independent risk factors that affect stress perceptions. The level of statistical significance was set at $p = 0.05$.

Results

The questionnaire was e-mailed to 1,094 dentistry students. Completed forms were received from 919 students, resulting in a response rate of 84%. Of the total sample, 81.6% of the participants were studying at state universities and more than half were female. A total of 35.3% of the participants were in their 3rd year of studies, whereas the rest were in their 2nd (22.6%), 4th (15.9%), 1st (13.3%), or 5th (12.9%) academic year (Table 1).

Table 2 presents the PSS-10 scores of the participants. The stress levels of female students were significantly higher than those of male students ($p = 0.0001$). The stress levels of students at state universities were significantly higher than those of students enrolled at private universities ($p = 0.034$). As opportunities to attend remote classes from home or dormitories decreased, the stress levels of the students significantly increased ($p = 0.0001$). In addition, as the level of support provided by lecturers to the students decreased, the students' stress levels increased significantly ($p = 0.0001$). The comparison of knowledge regarding how to open, modify and upload online documents between the stress groups indicated that the stress levels of students with a low level of technological knowledge were significantly higher than those of other students ($p = 0.0001$). As the stress levels increased, student preference for traditional education also significantly increased ($p = 0.042$). The stress arising from concerns that distance education is unsuitable for pre-clinical and clinical training was significantly high ($p = 0.0001$). The evaluation of the responses to the open-ended question reflected that the problems encountered by highly stressed students were mostly adaptation-related ($p = 0.011$).

The results of the logistic regression analysis are shown in Table 3. For the analysis, all the parameters identified as statistically significant in Table 2 were added to the model. The results confirmed that gender, type of university, support from lecturers, satisfaction with distance education, and adaptation-related obstacles were significant factors related to students' opinions regarding distance learning. Among all the predictor variables, gender was most significantly associated with high stress levels,

with female students experiencing high stress 2.6 times (95% CI: 1.8–3.9) more frequently than male students.

Discussion

The COVID-19 pandemic has affected the education of more than 770 million students around the world due to the almost complete closure of schools, universities and colleges.⁴ The most frequently encountered obstacles in the distance education models implemented during this period are technical, financial, adaptation-related, or pedagogical in nature.¹³ Additionally, the difficulties associated with practical classes have increased exponentially, as is the case for dentistry faculties, where pre-clinical and clinical training are crucial. Pre-clinical training has been provided to some extent, but clinical training has been suspended by most faculties.¹⁸ Deficiencies in theoretical, pre-clinical and clinical training, together with the fear of professional inadequacy, can evoke stress among dentistry students. The main purpose of our research was to evaluate the satisfaction and stress levels of dentistry students in Turkey regarding the distance education process compelled by the restrictions imposed by the COVID-19 crisis.

The use of questionnaires to evaluate the satisfaction and stress levels of dentistry students is a valid research method.^{9,13,19,20} Web-based questionnaires are reported to have lower response rates than paper-based questionnaires.²¹ To minimize the risk of bias in survey studies, researchers should achieve an optimum response rate of 70–80%²² – a level that was exceeded in the current study (84%). Another important requirement is an adequate sample size. In previous studies conducted in different countries, the sample sizes were as follows: 832²⁰; 70⁹; 200¹⁹; 123¹³; 450⁸; 230²¹; 310²³; and 92.²⁴ The participation rate in the present research study was high and the sample size was 919.

There are 3 popular stress measurement tools: the Stress Appraisal Measure (SAM); the Impact of Event Scale (IES); and the PSS. The PSS is the most commonly used scale.¹⁰ It was initially developed as a 14-item scale that evaluated a participant's perceptions of the stressful events and situations which occurred in the previous month. The 4-item PSS (PSS-4) and the PSS-10 were later developed as shorter versions of the original scale. In our study, the PSS-10 was chosen due to concerns that participants would feel bored with an instrument containing too many questions and become distracted. On the other hand, we did not think the PSS-4 was sensitive enough to measure the stress levels.

The literature indicates differences in general stress levels between genders, with females showing higher stress levels than males.²⁵ Varying experiences regarding the educational system during the pandemic induced different stress levels among female and male students in our study.

Table 2. Comparison of the PSS-10 scores according to demographic variables and students' opinions on distance education

Questionnaire items	PSS-10 scores			p-value
	low stress	moderate stress	high stress	
Gender				
– female	20 (23.8)	388 (59.2)	134 (74.4)	0.0001*
– male	64 (76.2)	267 (40.8)	46 (25.6)	
University type				
– state	67 (79.8)	524 (80.0)	159 (88.3)	0.034*
– private	17 (20.2)	131 (20.0)	21 (11.7)	
Academic year				
– 1 st	8 (9.5)	88 (13.4)	26 (14.4)	0.253
– 2 nd	19 (22.6)	140 (21.4)	49 (27.2)	
– 3 rd	27 (32.1)	240 (36.6)	57 (31.7)	
– 4 th	12 (14.3)	105 (16.0)	29 (16.1)	
– 5 th	18 (21.4)	82 (12.5)	19 (10.6)	
Preferred device for participating in distance education				
– cellphone	23 (27.4)	192 (29.3)	57 (31.7)	0.911
– computer	18 (21.4)	126 (19.2)	39 (21.7)	
– laptop	42 (50.0)	325 (49.6)	80 (44.4)	
– tablet	1 (1.2)	12 (1.8)	4 (2.2)	
My opportunities (devices, access to the Internet, etc.) to participate in distance education classes from my home or dormitory are sufficient.				
– strongly disagree	7 (8.3)	42 (6.4)	21 (11.7)	0.0001*
– disagree	11 (13.1)	101 (15.4)	37 (20.6)	
– uncertain	10 (11.9)	123 (18.8)	44 (24.4)	
– agree	27 (32.1)	299 (45.6)	57 (31.7)	
– strongly agree	29 (34.5)	90 (13.7)	21 (11.7)	
On which platform or application do you follow your training?				
– Microsoft Teams	7 (8.3)	84 (12.8)	25 (13.9)	0.188
– Zoom	25 (29.8)	179 (27.3)	40 (22.2)	
– Google Meet	9 (10.7)	80 (12.2)	13 (7.2)	
– other	43 (51.2)	312 (47.6)	102 (56.7)	
During the distance education process, lecturers responsible for classes supported you in solving your problems.				
– strongly disagree	3 (3.6)	31 (4.7)	18 (10.0)	0.0001*
– disagree	12 (14.3)	80 (12.2)	30 (16.7)	
– uncertain	16 (19.0)	218 (33.3)	76 (42.2)	
– agree	36 (42.9)	276 (42.1)	42 (23.3)	
– strongly agree	17 (20.2)	50 (7.6)	14 (7.8)	
I know how to open, modify and upload online documents.				
– strongly disagree	3 (3.6)	20 (3.1)	8 (4.4)	0.0001*
– disagree	9 (10.7)	52 (7.9)	19 (10.6)	
– uncertain	4 (4.8)	129 (19.7)	45 (25.0)	
– agree	38 (45.2)	359 (54.8)	78 (43.3)	
– strongly agree	30 (35.7)	95 (14.5)	30 (16.7)	
Which of the following best describes your preference for participating in classes?				
– traditional learning method (face-to-face)	22 (26.2)	219 (33.4)	62 (34.4)	0.042*
– online education	23 (27.4)	98 (15.0)	24 (13.3)	
– hybrid education	39 (46.4)	338 (51.6)	94 (52.2)	
The distance education process is suitable for dentistry pre-clinical and clinical practice training.				
– strongly disagree	50 (59.5)	457 (69.8)	148 (82.2)	0.0001*
– disagree	13 (15.5)	122 (18.6)	14 (7.8)	
– uncertain	7 (8.3)	45 (6.9)	7 (3.9)	
– agree	5 (6.0)	15 (2.3)	3 (1.7)	
– strongly agree	9 (10.7)	16 (2.4)	8 (4.4)	
I am satisfied with distance education.				
– very satisfied	14 (16.7)	21 (3.2)	8 (4.4)	0.0001*
– satisfied	18 (21.4)	94 (14.4)	11 (6.1)	
– neutral	24 (28.6)	166 (25.3)	28 (15.6)	
– dissatisfied	14 (16.7)	191 (29.2)	42 (23.3)	
– very dissatisfied	14 (16.7)	183 (27.9)	91 (50.6)	
What is the major problem you encountered in the distance education process?				
– technical obstacles	11 (13.1)	112 (17.1)	22 (12.2)	0.287
– financial obstacles	2 (2.4)	29 (4.4)	10 (5.6)	0.699
– adaptation obstacles	7 (8.3)	67 (10.2)	32 (17.8)	0.011*
– practical training obstacles	21 (25.0)	223 (34.0)	48 (26.7)	0.063
– pedagogical obstacles	21 (25.0)	153 (23.4)	45 (25.0)	0.935
– other	22 (26.2)	71 (10.8)	23 (12.8)	0.0001*

* statistically significant (χ^2 tests).

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

Table 3. Factors associated with the students' stress levels according to the PSS-10 scores

Factors associated with the students' stress levels	p-value	OR	95% CI for OR	
			lower	upper
Gender	0.000*	2.633	1.795	3.864
University type	0.046*	1.658	1.087	2.784
During the distance education process, lecturers responsible for classes supported you in solving your problems	0.007*	1.297	1.074	1.567
I am satisfied with distance education	0.000*	1.461	1.195	1.768
Adaptation obstacles	0.000*	2.440	1.500	3.960
Constant	0.000*	0.030	–	–

OR – odds ratio; CI – confidence interval; * statistically significant (logistic regression analysis).

The fact that male students exhibited lower stress levels in comparison with female students is accordant with the literature. However, we can only make suppositions as to the reasons for the considerably higher stress levels of female students. We attribute this phenomenon to the fact that female students do not feel as comfortable with technology as male students do.

When we evaluated the effect of type of university on differences in the stress levels, we found that the high stress levels of dentistry students from state universities were related to the limitations of these institutions and/or students. Most state universities were unprepared for the distance education process; they did not have sufficient infrastructure, which prevented them from organizing theoretical courses online. Although several months had passed since the onset of the pandemic, limited facilities (e.g., unsuitable clinical settings) hindered the initiation of clinical training at most state universities in Turkey. The better financial standing of private universities enabled these institutions to rapidly arrange the infrastructure required for distance education. Additionally, students from private universities had better opportunities (better devices, access to the Internet, etc.) to participate in distance education classes. Thus, they experienced lower stress than students from state universities.

Our study was conducted among students from various state and private universities in Turkey, and no significant differences in the stress levels were found between students in different academic years. We expected stress to increase as students progressed to a higher academic year. However, since dentistry education involves pre-clinical and clinical training from the 1st to the 5th year, the stress levels were similar across all academic levels. Previous studies involving dentistry students were conducted within just 1 academic term.^{5,24} In this respect, the current study was able to evaluate the issue more comprehensively, as it included dental students from all academic years.

Inquiring into the students' abilities to attend distance education classes from their homes or dormitories, we uncovered that those with insufficient opportunities suffered from significantly higher stress levels. The study also

ascertained the factors causing stress among the students by probing the most serious problem encountered during distance education. In this context, the main problem was the unsuitability of distance education as an avenue for practical dentistry training (pre-clinical and clinical). The participants thought that no virtual session could replicate the real experience with patients (practical training obstacles), which is similar to the findings of Iyer et al.²⁶ Other major obstacles faced by the participants were pedagogical, technical and adaptation-related challenges. Our results are also consistent with those of Rahali et al., who reported that the experience of distance education led to the emergence of many technical, psychological and/or pedagogical barriers.¹³

A recent work, likewise, investigated the advantages, limitations and suggestions associated with e-learning for both students and faculty members during the COVID-19 pandemic.²⁷ The participants indicated the inability of faculty members to teach psychomotor skills, resource overabundance and the mismanagement of the educational process as limitations of online learning. Whereas faculty members complained about a lack of feedback regarding students' understanding of a topic, students reported that their attention span during online learning was shorter as compared to face-to-face sessions.²⁷ A study by Bourzgui et al. also emphasized that most dentistry students had insufficient teacher–student interactions during online classes,²⁸ which supports the results of the above study.

In our study, one of the issues the students complained about was the insufficient interest of lecturers in distance education, or a complete lack of it. A study on the perceptions of online education among dentistry students before the pandemic revealed that most students (61.2%) were unsatisfied with the quality of the online lessons and procedural videos provided to them, highlighting the need for further improvements.⁹ High-quality online tutorials are an important success factor for establishing an effective online learning system. This condition can be addressed by providing training to lecturers on how to use online teaching/learning tools. Trainers should also adapt to rapidly developing technology.

Studies have revealed significant gender differences in attitudes toward technology. In general, men express more interest in technological innovations and exhibit more knowledge in this respect, while women report experiencing more difficulties and reduced interest in using technology.^{29,30} According to the logistic regression analysis in the present work, female students experienced more considerable stress than male students. The higher overall satisfaction of male students with e-learning and their lower stress levels might be related to the higher technological self-efficacy of these students. This finding confirms previous reports regarding the effects of lower perceived computer self-efficacy and higher computer anxiety among female students on their perceived satisfaction with distance education.²⁴

In other studies on distance education among dentistry students, students' perceptions of this educational delivery system were mostly negative.^{5,9,13} Similarly, the present research registered a very low percentage of dentistry students (18.1%) declaring satisfaction with distance education. Practical classes are critical for gaining professional competence in dentistry education and the insufficient amount of practice plays a substantial role in reducing students' satisfaction with distance education. In our study, the students identified practical training obstacles as the major problem they faced in distance education – a result in line with the literature. To address these concerns, the innovative platforms used for theoretical lectures (Zoom, Google Meet, etc.) can be integrated into practical training. Another valuable strategy is to share the recorded videos of laboratory simulations or demonstrations with dentistry students. Note that an equally important approach is to consider feedback from students regarding their understanding of the subject matter. In a study where video conferencing tools were combined with the simulated equipment, the feedback received from students was positive.³¹

Most of the students in Abbasi et al.'s work stated that they preferred face-to-face education,⁵ whereas most of our participants expressed preference for hybrid education over traditional learning. This difference can be ascribed to variations in the online provision of the practical training curriculum. In addition, it could be related to students' perceptions of the severity of the COVID-19 pandemic. We agree that hybrid teaching is the most suitable educational model during the pandemic for Generation Z students, who can easily use digital technologies and applications, and exhibit openness to technology. Faculty members should be educated in this regard to guarantee the effectiveness of distance education; we believe that such training can be useful in the delivery of theoretical and practical classes.



Considering the unexpected changes stemming from the pandemic, the findings of our study show that an important task in dentistry education is to continue to teach quality and adequate theoretical content. More

systematic teaching methods regarding clinical practice can be developed by making changes to the curricula, and more research should be conducted to illuminate how distance dentistry education can be enhanced. This work is limited in that it did not compare the effectiveness of distance learning and face-to-face education. Further studies should evaluate the 2 modes of educational delivery in this regard.

Conclusions

Our findings showed that the abrupt closure of universities due to the COVID-19 pandemic had prompted enormous transformations in the dentistry education system. Dentistry students were concerned that their pre-clinical and clinical training would be insufficient under distance education conditions, and correspondingly struggled with increased stress levels. Gender affected stress, with female students experiencing greater stress than male students. The results also reflected the importance of support from lecturers in the distance education process. Finally, the students were generally unsatisfied with the distance education process.

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Effect of teledentistry on the oral health status of patients undergoing fixed orthodontic treatment at the first three follow-up visits

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Abstract

Background. As the prevalence of such oral diseases as dental caries and periodontal problems increases during fixed orthodontic treatment, patient education is an essential aspect of any such treatment. Two methods have been proposed for this purpose: direct education in a clinic (the conventional method); and education using social media, such as the Telegram application (a type of teledentistry).

Objectives. The aim of this study was to evaluate the effect of teledentistry as an educational tool on the oral health status of patients undergoing fixed orthodontic treatment at the first 3 follow-up visits.

Material and methods. Sixty participants were enrolled from patients whose fixed orthodontic treatment was to begin at a dental clinic in Tehran, Iran. They were randomly assigned to one of 2 trial arms. At the end of the 1st appointment, one of the groups was educated in person about maintaining oral hygiene during treatment, and an educational clip was sent to the members of the other group via the Telegram application. During the next 3 follow-up appointments, plaque index (PI), bleeding on probing (BOP), and gingival color and consistency were analyzed for each patient to assess their oral hygiene compliance during treatment.

Results. There was a statistically significant difference in PI and BOP between the 2 groups at the 3rd and 4th appointments; however, gingival color and consistency did not differ significantly with regard to the manner of education ($p > 0.05$). Patient age did not have a considerable effect on the oral health status in either group ($p > 0.05$).

Conclusions. Teledentistry is an effective and efficient method to improve oral hygiene in patients undergoing fixed orthodontic treatment.

Keywords: orthodontics, telemedicine, mobile health, remote consultation

Introduction

What is teledentistry?

Teledentistry is a type of telemedicine that uses information technology and telecommunication to provide access to dental care and consultation with specialists as well as to increase public awareness regarding oral health.^{1,2} This method can provide new opportunities for patients and clinicians to improve the quality of treatment.³ Moreover, teledentistry can be used as an approach for screening dental diseases, for patient education through conversation and videos, and patient referral for advanced dental treatment.⁴ Many studies have described the ease and reliability of this technology-based approach (with the aid of technologies like smartphones)² as compared to the traditional methods.⁵ For example, in a study conducted by Alipour-Rocca et al., evaluating the ability of mothers to diagnose caries by means of teledentistry, it was concluded that mothers were able to diagnose caries through smartphone-based photographs.⁶ Teledentistry is not only used to increase access to dental care, but it can also be used for advanced dental education.⁵ Chen et al. stated in their study that teledentistry could extend care to people in distant locations, such as those in rural areas, with a reasonable cost and the aid of telecommunication technologies.⁷ Another study reported that teledentistry increased the quality of oral healthcare provided to children living in remote rural areas of California, USA.⁸

Exacerbation of dental diseases associated with fixed orthodontic treatment

Dental caries and periodontal problems may be exacerbated during fixed orthodontic treatment due to the lack of proper education. In this regard, one reason can be the lack of skilled practitioners in remote areas, and another can be the inability to refer patients to advanced dental healthcare systems due to geographic or financial limitations. In addition, patients undergoing fixed orthodontic treatment present changes in the oral cavity, such as a decrease in salivary pH, the creation of additional food trap locations and increased levels of *Streptococcus mutans*.⁹ Nowadays, fixed orthodontic appliances are the most common devices for orthodontic treatment.¹⁰ It has been shown that the placement of bands and brackets leads to poor oral hygiene and an increased plaque index (PI).¹¹ Since these appliances make controlling microbial plaque (the main cause of periodontal inflammation) more difficult, oral hygiene should be carefully monitored during the performance of orthodontic treatment.^{11,12} Plaque index and bleeding on probing (BOP) are the 2 main assessments used to interpret the oral hygiene status.¹³

Objectives

Based on the importance of oral hygiene in patients with fixed orthodontic appliances and the wide use of telecommunication via the Internet in healthcare education, this study aimed to evaluate the effect of teledentistry on the oral health status of patients undergoing fixed orthodontic treatment at the first 3 follow-up appointments.

Material and methods

This interventional study was designed with 60 participants selected from patients who were starting fixed orthodontic treatment in a dental clinic in Tehran, Iran, during the summer of 2019. Only patients with permanent dentition, class I dental and skeletal relationships with mild to moderate tooth size discrepancy, no need for extraction, no history of previous orthodontic treatment, no active medical or dental diseases, regular access to a smartphone, and willingness and informed consent to participate were included in this study.

All patients were treated using similar techniques and materials by a trained orthodontist. The participants were randomly divided into 2 groups while they were blinded to the group assignment. Each group was then divided into 2 subgroups based on the patients' age to investigate the effect of this parameter on the oral health status while performing orthodontic treatment. Patients aged 12–18 years ($n = 12$) and patients older than 18 years ($n = 18$) were included in both trial arms.

Treatment and intervention sequences

Before banding and bonding, the patients underwent a session of oral hygiene measurements to obtain a PI of 0. Then, they were examined in terms of BOP, gingival color (pink) and gingival consistency (firm) of the upper and lower arches (from right to left upper first molar and from left to right lower first molar). Of note, if there was any evidence of active periodontal disease, the participant was excluded.

The treatment sequence during the 1st appointment consisted of banding all first molars, and bonding all upper and lower anterior and premolar teeth. Thereafter, a 0.012-inch nickel-titanium (Ni-Ti) wire was inserted; it was retied during the 2nd appointment to relieve crowding more efficiently. During the 3rd appointment, a 0.014-inch Ni-Ti wire was put in place for the next 4 weeks.

For the control group, at the end of the 1st appointment, an operator thoroughly explained the importance of oral hygiene during orthodontic treatment for about 5 min. Thereafter, she used a dental model to instruct the participants precisely on the Bass brushing technique (the preferable technique for orthodontic patients) and the use of interdental brushes. She showed the replaceable tips of the brushes,

which are used with no toothpaste, and are gently moved back and forth in the interdental spaces. She suggested using them in addition to regular tooth brushing twice a day – after lunch and before bedtime. Then, she recommended flossing at least once a day before bedtime with orthodontic dental floss due to its stiff head, which makes it easier to thread the floss under the archwire. She demonstrated threading plaque away from the gums. Finally, she explained the importance of using mouthwash and suggested rinsing 3 times a week at night, for 2–3 min each night.

In the teledentistry group, after the 1st appointment, the participants received a video clip of a duration of 5 min and 7 s via the Telegram application. In the video, the same operator educated the participants about oral healthcare methods in a manner similar to the way the control group was instructed.

Plaque index, BOP, gingival color, and gingival consistency were recorded for each participant during the next 3 appointments at 4-week intervals. Finally, the collected data was entered into a checklist and subjected to statistical analysis.

Ethical considerations

The participants were fully informed and volunteered to participate in the study. Each participant's checklist information was completely confidential, and each participant was assigned a code. Ethical approval was obtained for this research from the Dental Research Center at Qom University of Medical Sciences in Iran (IR.MUQ.REC.1398.058).

Statistical analysis

The data was analyzed using the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA). Differences in the mean PI and BOP scores between the control and intervention groups were evaluated using the independent *t* test. Differences in the PI and BOP scores within each group were evaluated using the repeated analysis of variance (ANOVA). Afterward, the χ^2 test was used to compare the mean changes in gingival color and consistency at each appointment. It was also used to evaluate the effect of patient age on the oral hygiene status during the course of treatment. Friedman's test was used to calculate the significance of changes in gingival color and consistency during the study. The level of statistical significance was set at 0.05.

Results

Plaque index and bleeding on probing

As shown in Table 1 and Table 2, the mean PI and BOP scores were similar for both groups at the 2nd appointment; however, the teledentistry group had

decreased PI and BOP levels at the 3rd and 4th appointments. On the contrary, the control group showed increased levels of these 2 indices at the 3rd and 4th appointments.

Table 1. Comparison of the mean plaque index (PI) scores between the intervention and control groups at 3 follow-up visits

Appointment	Group	PI	<i>p</i> -value
2 nd	teledentistry	36.12 ±19.40	0.820
	control	37.30 ±19.93	
3 rd	teledentistry	32.82 ±18.21	0.010*
	control	46.45 ±22.11	
4 th	teledentistry	24.91 ±12.76	0.000*
	control	59.53 ±14.65	

* statistically significant.

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

Table 2. Comparison of the mean bleeding on probing (BOP) scores between the intervention and control groups at 3 follow-up visits

Appointment	Group	BOP [%]	<i>p</i> -value
2 nd	teledentistry	26.20 ±20.63	0.680
	control	24.21 ±16.70	
3 rd	teledentistry	23.40 ±15.73	0.020*
	control	36.00 ±23.24	
4 th	teledentistry	19.47 ±14.21	0.004*
	control	67.67 ±85.64	

* statistically significant.

Data presented as *M* ±*SD*.

Gingival color

As shown in Table 3, except for the 3rd visit, during which there was no significant difference noticed between the 2 groups, the percentage of participants with coral pink gingivae was higher for the control group at the 2nd appointment and higher for the teledentistry group at the 4th appointment, with both differences statistically significant.

Table 3. Comparison of gingival color between the intervention and control groups at 3 follow-up visits

Appointment	Group	Participants with coral pink gingivae (%)	<i>p</i> -value
2 nd	teledentistry	33.3	0.020*
	control	63.3	
3 rd	teledentistry	53.3	0.440
	control	43.3	
4 th	teledentistry	56.7	0.020*
	control	26.7	

* statistically significant.

Gingival consistency

This index was significantly different between the intervention and control groups at the 3rd and 4th appointments ($p \leq 0.05$), and the differences were in favor of the teledentistry group. However, no significant difference was found at the 2nd visit. Details are presented in Table 4.

According to the results of Friedman's test, there was no significant difference between the intervention and control groups in terms of gingival color and consistency during the study ($p > 0.05$).

Table 4. Comparison of gingival consistency between the intervention and control groups at 3 follow-up visits

Appointment	Group	Participants with firm and elastic gingivae	<i>p</i> -value
2 nd	teledentistry	60.0	0.590
	control	66.7	
3 rd	teledentistry	86.7	0.037*
	control	63.3	
4 th	teledentistry	80.0	0.001*
	control	36.7	

* statistically significant.

Effect of age

In both the intervention and control trial arms, 40% of the patients were between 12 and 18 years of age, and 60% were above 18 years of age. There was no significant difference between these 2 subgroups in terms of PI, BOP, gingival color, and gingival consistency during the study based on the χ^2 test ($p > 0.05$).

Discussion

The evaluation of the efficacy of different educational techniques during dental treatment procedures may decrease the prevalence of such oral diseases as dental caries and periodontal problems. This study was conducted to investigate the efficacy of teledentistry as a relatively new telecommunication method for instructing patients undergoing fixed orthodontic treatment about oral hygiene. The results indicated that this method could have a considerable positive effect on the patients' oral health status during the first 3 months after starting treatment. The patients' knowledge about using new educational methods was improved, which might lead to the improved oral hygiene, and the reduced cost and pain burden for both the patients and the society.

In a previous study that evaluated the efficacy of a mobile phone-based application in teaching preventive dental care to the mothers of children under the age of 6 years, Hebbal et al. found that the mobile phone

application significantly improved the mothers' knowledge about their children's oral health.¹⁴ Shah et al. assessed the improvement of oral health knowledge after exposure to an educational video in a hospital setting and reported a statistically significant increase in the knowledge scores.¹⁵ According to studies by Birang et al.¹⁶ and Darby and Walsh¹⁷ on using visual media to promote oral hygiene, visual media could improve oral health-related knowledge and practice, both in the short and long term. In addition, they could provide instructions in a faster and superior way.^{16,17}

Plaque index

As a major indicator of the oral health status, PI decreased over the course of treatment in the teledentistry group. Notably, there was no significant difference in PI between the 2 groups at the 2nd visit, but this index decreased in the teledentistry group at the 3rd and 4th appointments. Conversely, PI actually increased in the patients who had only been instructed in person. The reason for increased PI scores in the control group may be the fact that the participants had no access to an educational source like the video clip to be used as a guide at home. Therefore, they may have forgotten the instructions they had learned during their very first appointment. It is noteworthy that searching Internet sources is an easy way to access unlimited images, clips and websites with oral hygiene instructions during orthodontic treatment; however, many patients lack the time and/or interest to conduct a web search. As a result, deteriorating oral hygiene during fixed orthodontic treatment is a common consequence of using conventional education methods only. It should also be kept in mind that in the case of conventional education, the excessively frequent repetition of the topic may lead to a more negative attitude as well as decreased patient cooperation.¹⁸

Hebbal et al. evaluated the oral hygiene-related knowledge and PI scores of 12-year-old schoolchildren after health education with audiovisual aids as compared to control (with no education at all) and conventional education (chalk and blackboard) groups.¹⁴ After performing advanced intervention, the mean knowledge scores increased more in the trial group as compared to the conventional education and control groups. Although the mean PI scores decreased significantly in all 3 groups, the decrease was more evident when audiovisual aids were used as the educational method.¹⁴ Additionally, in accordance with the results of the abovementioned study, Albandar et al. found that the PI scores improved in a group of children who underwent a comprehensive education program in contrast to a control group.¹⁹ Other comparable studies also concluded that PI could be significantly improved after educational courses.^{20–23} While Najjari et al. reported lower PI scores after patient education, they found no statistically significant difference between intervention and

control (with conventional education) groups in terms of this index.²⁴ In contrast to our findings, Ghorbani reported increased PI levels in a group that underwent education with a movie.²⁵ The author concluded that the conventional method of patient education is still the most logical and reliable way to improve the oral hygiene status.²⁵ This difference may be related to the quality of instructions in the movie as well as the patients' desire and interest to learn from the educational movie.

Bleeding on probing

Similar to the PI scores, the mean scores of the BOP index were similar for the 2 groups at the 2nd appointment, but then gradually decreased in the teledentistry group and increased in the control group. In line with these findings, 2 other studies showed decreased BOP levels after performing an intervention in the form of an educational video.^{24,26} However, in contrast to the results of the present investigation, there was no significant difference between the mean BOP scores of trial and control groups.^{24,26} Another study regarding BOP changes following an educational program (the WHO Health Promoting Schools project) showed a significant decrease in BOP levels after oral hygiene education was provided to schoolchildren.²⁷

Gingival color and consistency

The results of this study indicate that some negative changes in gingival color and consistency occurred in both groups, but these changes were less prominent in the patients educated by means of teledentistry. This means that gingival health was preserved more in this group. In this regard, more investigations are needed to reach a definite conclusion.

Based on the results of this study, and since teledentistry is quite a new method for screening and improving the oral health status during fixed orthodontic treatment, more studies are required to establish definite guidelines and to make teledentistry a routine part of clinical orthodontic practice.

Conclusions

Teledentistry, which in this study meant sending an educational movie to patients using smartphones and a telecommunication application, can be considered a useful method to decrease plaque accumulation and gingival bleeding in patients undergoing fixed orthodontic treatment. The possibility of watching the educational movie more than once at home can act as a reminder, which may consequently improve the patient's cooperation. However, the results of this study should be interpreted with caution because of the limited number of investigations performed in this field.

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Pain assessment following endodontic treatment using two automated systems compared to manual treatment in primary molars

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Abstract

Background. Root canal treatment in primary teeth is quite difficult and time-consuming, especially canal preparation. Pain is the most common negative outcome following root canal treatment, occurring hours or days after treatment. It is an unpleasant experience for both the patient and the dentist.

Objectives. The objective of this study was to assess the severity and duration of pain following the endodontic treatment of primary molars with the use of rotary and reciprocating preparation systems compared to the traditional manual method.

Material and methods. The research sample consisted of 157 asymptomatic primary lower second molars with non-vital pulp that were indicated for root canal treatment. The patients were randomly divided into 3 groups: in the 1st group, the molars ($n = 52$) were prepared using the manual method; in the 2nd group ($n = 53$), a reciprocating automated preparation system (WaveOne[®]) was used; and in the 3rd group ($n = 52$), a rotary preparation system (ProTaper Next[®]) was used. Pain assessment was carried out after 6, 12, 24, 48, and 72 h, and after 1 week, using a four-face facial pain scale.

Results. The manual method provided a higher pain score through 6, 12 and 24 h ($p < 0.05$) as compared to the 2 automated preparation systems. The intensity of pain did not vary between the 2 machine preparation systems after 6, 12, 24, and 48 h ($p > 0.05$). There were no differences between the 3 methods after 72 h and after 1 week ($p > 0.05$).

Conclusions. The manual method caused more pain than the other 2 preparation systems, but there was no difference between the 2 automated methods. Automated root canal preparation systems could be used to reduce the intensity of postoperative pain after the endodontic treatment of primary teeth.

Keywords: ProTaper Next, rotary system, reciprocating system, WaveOne, deciduous molars

Introduction

Root canal treatment in primary teeth is quite difficult and time-consuming, especially canal preparation. Despite the complicated anatomical form of complex molar roots, root canal treatment must be considered an essential option, especially in cases of pulpitis.¹

Pain with or without edema is the most common adverse effect of root canal treatment. It can occur hours or days after treatment, and is an unpleasant experience for both the patient and the dentist. Debris extruded from the apical foramen during canal preparation can induce an acute inflammatory response, which leads to post-operative pain.²

In 2000, Barr et al. described the advantages of using rotary instrumentation techniques for the preparation of primary teeth.³ Many studies have concluded that automated systems significantly reduce chairside time and are able to maintain the anatomical form of primary molar roots while reducing the errors that can occur when using the manual system, such as instrument fracture.^{3,4} Nickel-titanium (NiTi) files have been found to be more flexible, allowing easy access to all canals and reducing instrument failure in comparison with K-file hand instrumentation, which increases efficiency in terms of preparation time.⁴

A rotary system (ProTaper Next®; Dentsply Sirona, Ballaigues, Switzerland) with a distinctive design, made of M-Wire NiTi alloy to enhance the flexibility and cyclic fatigue resistance of the files was introduced in 2000.⁵

Studies have investigated the use of a reciprocating system (WaveOne®; Dentsply Maillefer, Ballaigues, Switzerland) to shape root canals.⁶ Primary reciprocating files use a pecking motion as an alternative to a rotational motion in the preparation of curved canals with a single file. This technique enables preparing canals safely, with better irrigation and less dentin being removed from the inner walls of the root canal.⁷

WaveOne is a system that applies an alternating motion in preparation with a single file of 3 sizes: small (21/06); primary (25/08); and large (40/08).⁸

To our knowledge, few studies have assessed post-operative pain following the endodontic treatment of primary molars with the use of rotational and alternating automated regimens compared to the manual system.^{9–11} Therefore, the purpose of this study was to assess post-operative pain after the canal instrumentation of primary molars with the WaveOne and ProTaper Next preparation systems compared to the traditional manual method.

Material and methods

The sample size was calculated using G*Power software (<https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower>)

based on a previous study,⁹ where the study strength was 0.91 with a 5% level of significance. The sample size required for each group was determined to be 50 patients. Five patients were added to each group in anticipation of withdrawal from the study, giving the total number of 55 patients in each group.

The study was approved by the Ethics Committee of Damascus University, Syria (the approval No. 1438). Informed consent was obtained from each child's parents after a thorough explanation of the study protocol and treatment requirements.

The inclusion criteria were as follows: cooperative children aged 5–8 years; absence of physical or mental disability that would prevent the child from understanding treatment instructions; presence of pulp necrosis caused by carious exposure in primary second molars with at least 2/3 of the root length present, with no signs of fistulae, swelling or pathological tooth mobility; and no evidence of pathology on radiological images (bifurcation lesions, apical lesions or periapical abscesses).

The exclusion criteria were as follows: patients with immune or systemic diseases; patients who took painkillers 12 h before treatment; and patients who refused to participate in the study.

The patients were distributed randomly into study groups by asking them to select a ball from a dark box that contained 3 balls numbered from 1 to 3; the balls determined the patient's study group number.

The treatment was completed in 1 session. It was performed by one dentist according to the following method:

- application of local anesthetic (2% lidocaine with 1:200,000 adrenaline);
- isolation of the tooth with a rubber dam;
- removal of caries followed by opening the pulp chamber with cooling water while making sure the pulp was completely non-vital and that there was no hemorrhage in the canals;
- determination of the canal length with an apex locator so that the final length was 1 mm shorter than the point 0.0 indicated on the device (the final length was confirmed with an X-ray);
- irrigation of the canal with 5 mL of 1% sodium hypochlorite solution after using each file with a syringe and a 29-gauge double sideport NaviTip™ irrigation needle (Ultradent Products Inc., South Jordan, USA).

The research sample ultimately consisted of 157 asymptomatic primary lower second molars with non-vital pulp. The study groups were prepared in the following order:

- group 1 ($n = 52$) (manual method): The balanced force technique was used. The mesial canals were instrumented manually with K-files (Dentsply Maillefer) 15/02, 20/02 and 25/02 used sequentially. The distal canals were instrumented with files 15/02, 20/02, 25/02, 30/02, and 35/02, applying irrigation after using each file;

- group 2 ($n = 53$) (WaveOne system): All canals were prepared with small file (21/06) instrumentation in accordance with the manufacturer's instructions;
- group 3 ($n = 52$) (ProTaper Next system): The mesial canals were prepared with ProTaper files X1 (17/04) to expand the coronal part of the canal and X2 (25/06) to achieve the full working length. The distal canals were prepared with file X1 to expand the coronal part, and files X2 and X3 (30/07) to achieve the full working length. Brushing movements were performed gently with an in-and-out motion in accordance with the manufacturer's instructions (rotational speed: 300 rpm; torque: 200 gcm), applying irrigation after using each file.

Each file was used only once and there were no complications associated with any technique.

After that, each canal was washed with 2 mL of distilled water, dried with paper pins, and then injected with a combination of calcium hydroxide with iodoform (Metapex; Meta Biomed Co. Ltd., Cheongju, South Korea). Finally, the teeth were restored with stainless steel crowns (Kids Crown; Shinhung Co. Ltd., Seoul, South Korea).

After the treatment was completed, the parents were given instructions about how to use the four-face pain scale denoted by numbers: 0 – no pain; 1 – slight pain; 2 – moderate pain; and 3 – severe pain (Fig. 1).

The parents were given a pain relief prescription in case of severe pain (ibuprofen or paracetamol if there was a contraindication to ibuprofen).

The numbers were recorded after communication by telephone with the parents, who did not know the working protocol. They reported to the doctor at the following points in time: 6, 12, 24, 48, and 72 h, and 1 week after treatment.

Statistical analysis

The Mann–Whitney and Kruskal–Wallis tests were used to assess the severity and duration of pain, and to study differences between the groups. The IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA) was used for data analysis. The results were considered to be statistically significant at $p \leq 0.05$.

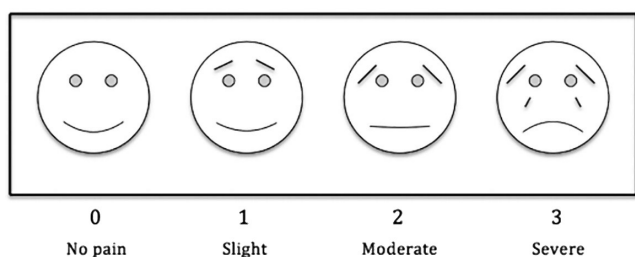


Fig. 1. Pain scale used in the study

Results

The study began with 165 patients in 3 groups. Eight patients were excluded from the study due to our inability to contact them after the treatment. In the 1st group, the molars ($n = 52$) were prepared using the manual method. In the 2nd group ($n = 53$), a reciprocating automated preparation system (WaveOne) was used. In the 3rd group ($n = 52$), a rotary preparation system (ProTaper Next) was used.

Table 1 details the distribution of patients in the study groups with their gender and average age. Table 2 presents the descriptive pain scores in the 3 study groups. The severity of pain and differences between the 3 groups at the time points 6, 12, 24, 48, and 72 h, and 1 week after the treatment were recorded (Table 3). Table 4 provides the test results of Mann–Whitney pairwise comparisons at 6, 12 and 24 h. No patients manifested severe pain requiring analgesia.

The pain intensity was higher in the 1st group (manual preparation group) as compared to the 2 automated preparation system groups at the time points 6, 12 and 24 h ($p < 0.05$), and 48 h ($p > 0.05$) after the treatment. There were no differences between the 3 groups at 72 h and 1 week after the treatment ($p > 0.05$). It was observed that there were no differences between the 2 machine systems at the time points 6, 12, 24, 48, and 72 h, and 1 week after the treatment ($p > 0.05$).

Table 1. Gender distribution and average age of the patients

Patients' characteristics	Group		
	hand files	WaveOne	ProTaper Next
Males <i>n</i>	27	29	30
Females <i>n</i>	25	24	22
Age [years] <i>M</i> ± <i>SD</i>	6.80 ± 0.50	6.95 ± 0.90	7.84 ± 0.64

M – mean; *SD* – standard deviation.

Table 2. Descriptive pain scores in the 3 study groups

Assessment time	Hand files <i>n</i> = 52			WaveOne <i>n</i> = 53			ProTaper Next <i>n</i> = 52		
	<i>M</i>	<i>Me</i>	<i>SD</i>	<i>M</i>	<i>Me</i>	<i>SD</i>	<i>M</i>	<i>Me</i>	<i>SD</i>
6 h	1.15	1.00	0.78	0.72	1.00	0.72	0.69	1.00	0.76
12 h	0.85	1.00	0.83	0.64	1.00	0.71	0.56	0.00	0.73
24 h	0.60	0.00	0.75	0.42	0.00	0.66	0.40	0.00	0.66
48 h	0.48	0.00	0.75	0.30	0.00	0.57	0.29	0.00	0.61
72 h	0.12	0.00	0.32	0.08	0.00	0.27	0.08	0.00	0.27

Me – median.

Table 3. Distribution of the patients from the 3 study groups in terms of pain severity and duration (Kruskal–Wallis test)

Assessment time	Pain	Hand files (n = 52)	WaveOne (n = 53)	ProTaper Next (n = 52)	p-value
6 h	none	12 (23.1)	23 (43.4)	25 (48.1)	<0.05*
	slight	20 (38.5)	22 (41.5)	18 (34.6)	
	moderate	20 (38.5)	8 (15.1)	9 (17.3)	
	severe	0 (0)	0 (0)	0 (0)	
12 h	none	22 (42.3)	26 (49.1)	30 (37.7)	<0.05*
	slight	16 (30.8)	20 (37.7)	15 (28.8)	
	moderate	14 (26.9)	7 (13.2)	7 (13.5)	
	severe	0 (0)	0 (0)	0 (0)	
24 h	none	29 (55.8)	36 (67.9)	36 (69.2)	>0.05
	slight	15 (28.8)	12 (22.6)	11 (21.2)	
	moderate	8 (15.4)	5 (9.4)	5 (9.6)	
	severe	0 (0)	0 (0)	0 (0)	
48 h	none	35 (67.3)	40 (75.5)	41 (78.8)	>0.05
	slight	9 (17.3)	10 (8.9)	7 (13.5)	
	moderate	8 (15.4)	3 (5.7)	4 (7.7)	
	severe	0 (0)	0 (0)	0 (0)	
72 h	none	46 (88.5)	49 (92.5)	48 (92.3)	>0.05
	slight	6 (11.5)	4 (7.5)	4 (7.7)	
	moderate	0 (0)	0 (0)	0 (0)	
	severe	0 (0)	0 (0)	0 (0)	
1 week	none	0 (0)	0 (0)	0 (0)	–
	slight	0 (0)	0 (0)	0 (0)	
	moderate	0 (0)	0 (0)	0 (0)	
	severe	0 (0)	0 (0)	0 (0)	

* statistically significant.

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

Table 4. Pairwise comparisons between the 3 study groups as a post-hoc test at 6, 12 and 24 h (Mann–Whitney test)

Group pairs	p-value
Hand files vs WaveOne	<0.05*
Hand files vs ProTaper Next	<0.05*
WaveOne vs ProTaper Next	>0.05

*statistically significant.

Discussion

Pain associated with root canal treatment is a major source of fear and a very important concern for patients. Studies have found a postoperative pain rate of 1.9–48.9%,

which causes anxiety in patients.^{12,13} Therefore, finding the best instrumentation techniques that cause the least amount of pain after root canal treatment is an essential objective of clinical practice. To our knowledge, few studies have compared postoperative pain following the endodontic treatment of primary molars with the use of rotational and alternating automated regimens compared to the conventional manual system.^{9–11}

Thus, the purpose of this study was to assess the intensity of pain following the root canal treatment of primary molars with 2 automated (rotary and reciprocating) preparation systems compared to the conventional manual system at the time points 6, 12, 24, 48, and 72 h, and 1 week after the treatment. The study involved asymptomatic primary second molars with non-vital pulp to avoid bias resulting from the tooth type and the pulp

condition. Sodium hypochlorite solution (1%) was used as an irrigation solution in accordance with the recommendations of the American Society of Pediatric Dentistry, as it is not recommended to use a high concentration of sodium hypochlorite solution in children.¹⁴

An apex locator was used to determine the working length. Many studies have confirmed the ability of the apex locator to determine the working length, even in the presence of resorption.^{15,16} The facial pain scale implemented in many studies was used to assess pain severity in this study.^{9,10,17}

During the mechanical and chemical preparation of root canals, debris (including dentinal particles, the irrigation solution, necrotic pulp tissues, and microorganisms) is propelled into the apical foramen, which may lead to an inflammatory response in the area around the apex and postoperative pain; it may also cause harm to the permanent tooth bud.²

The results of this study determined that there was post-treatment pain in all 3 groups, but pain intensity was higher in the manual preparation group as compared to the 2 automatic preparation systems at 6, 12 and 24 h ($p < 0.05$), which is consistent with a study by Topçuoğlu et al.⁹ Conversely, there were no differences in pain perception between the 3 groups at 72 h and 1 week post-treatment ($p < 0.05$), which is consistent with the results of studies conducted by Topçuoğlu et al.⁹ and Nair et al.¹⁰ However, it should be noted that their comparison was limited to only 2 preparation systems (manual and rotary).

Jeevanandan et al. concluded that there was more postoperative pain due to NiTi K-flex files used in a reciprocating motion followed by manual NiTi K-flex files and Kedo-S pediatric rotary files,¹¹ which does not match our findings. This could be due to differences in the tooth types treated and the systems used for preparation.

Our results are consistent with a systematic review by Manchanda et al., who found that there was less post-treatment pain while using an automated preparation system at the time points 6 h ($p < 0.05$) and 48 h ($p > 0.05$) when compared with a manual system.¹⁸ Also, no difference in pain intensity between manual and rotary systems at the 72 h point was found.¹⁸ Conversely, our study concluded that the use of the manual preparation system led to an increase in pain intensity 12 and 24 h after the treatment ($p < 0.05$), whereas Manchanda et al. stated that the levels of pain were similar for the 2 systems (manual and automated).¹⁸

It is speculated that a manual system propels a greater quantity of debris toward the region around the apex, which translates into greater pain as compared to the 2 automated preparation systems (rotary and reciprocating). This is consistent with the results of a laboratory study by Alnassar et al.¹⁹

Many factors could affect the results of the present study, e.g., the preparation technique, the direction of preparation or the characteristics of the preparation


tools used. For example, the files of the automatic preparation system ProTaper Next have a distinctive design – an offset center of mass and rotation. The files of this system are based on the crown-down technique, which relies on early coronal expansion. This pushes the preparation residue further into the coronal direction of the canal and reduces thrust in the apical direction.^{20,21} Conversely, the WaveOne system files have a convex, triangular cross-section, with the tip portion having a modified convex, triangular cross-section.⁶ The WaveOne system is based on an alternating movement in preparation, which is a type of balanced force that provides control over the movement of the residue, reducing its thrust in the apical direction.²² Manual files, on the other hand, tend to apply a pushing movement acting as a piston in the apical third of the canal, which propels the residue further, and thereby causes more pain.²³


Conclusions

The manual method caused more pain than the other 2 preparation systems, but there was no difference in terms of pain caused between the 2 automated methods. Automated root canal preparation systems could be used to reduce the intensity of postoperative pain in the endodontic treatment of primary teeth.


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Epithelial–mesenchymal transition in gingival tissues from chronic periodontitis patients: A case–control study

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Abstract

Background. It has been proposed that epithelial–mesenchymal transition (EMT) is responsible for the pathogenesis of several diseases. However, the relationship between the EMT process and the severity of periodontitis has not been previously investigated.

Objectives. This study aimed to localize and quantitatively assess the expression of transforming growth factor-beta 1 (TGF-β1), vimentin and E-cadherin in correlation with the EMT process in human gingiva of periodontally diseased patients in comparison with healthy individuals.

Material and methods. Gingival tissue samples from 36 participants were divided into 2 groups: the healthy (control) group ($n = 9$); and the periodontitis group ($n = 27$). The periodontitis group was further subclassified into mild, moderate and severe periodontitis subgroups (9 patients in each subgroup). The samples were subjected to histological staining, the histomorphometric analysis and the quantitative real-time polymerase chain reaction (RT-PCR) analysis for TGF-β, vimentin and E-cadherin. Statistical and correlation analyses were performed.

Results. The hematoxylin and eosin (H&E) stain sections from both the moderate and severe periodontitis subgroups showed epithelial hyperplasia, perinuclear haloing and a marked increase in the inflammatory cell count as compared to the control group. The highest mean TGF-β1 and vimentin expression values were recorded in the severe periodontitis subgroup, whereas the lowest mean values were recorded in the control gingiva. On the contrary, the expression of E-cadherin had the highest mean value in the control gingiva, whereas the lowest mean value was recorded in the severe periodontitis subgroup. All results were found to be statistically significant. The correlation analysis revealed a statistically significant positive correlation between the severity of periodontitis and the expression of TGF-β and vimentin, while a statistically significant inverse correlation was found between the expression of E-cadherin and the severity of periodontitis.

Conclusions. There is a direct correlation between the severity of periodontitis and the expression of the EMT process markers (TGF-β and vimentin). This correlation indicates that EMT plays an important role in the pathogenesis and prognosis of periodontal disease. The data presented in this study could open the door for using anti-EMT agents in treating periodontal disease.

Keywords: periodontitis, transforming growth factor-beta, vimentin, E-cadherin, epithelial–mesenchymal transition

Introduction

Periodontitis is an epidemic disease that affects a high percentage of the population. According to a systematic review from 2018, an estimated 42% of dentate US adults at the age of 30 years or older had periodontitis, with 7.8% having severe periodontitis; severe periodontitis was most prevalent among adults aged 65 years or older, Mexican Americans, non-Hispanic Black Americans, and smokers.¹ A study published in 2019 found that in Egypt, the prevalence of periodontitis was 89.8%; 70.8% of participants had stage I and 15.2% had stage II, while only 4.4% and 2.1% suffered from stage III and stage IV, respectively.²

Periodontitis encompasses a group of inflammatory conditions that affect the supporting tissues of the dentition. The disease is characterized by an aberrant host response in susceptible patients to the plaque biofilm, which consists of Gram-negative anaerobic bacteria.³ Hernández et al. reported that these pathogens could stimulate host cells, including the junctional epithelium (which is closely related to the plaque biofilm), to secrete a range of pro-inflammatory cytokines involved in initiating the epithelial–mesenchymal transition (EMT) process.⁴ It has been proposed that the EMT process and the resulting shift from an epithelial to mesenchymal phenotype could result in the loss of the integrity of the epithelial barrier, which would allow bacterial invasion into the underlying tissues and predispose the patient to periodontal disease.³

Epithelial–mesenchymal transition is a biological process that causes the polarized epithelial cells to undergo multiple biochemical changes to assume a mesenchymal cell phenotype.⁵ There is growing evidence that EMT plays important roles in embryogenesis, inflammation and cancer metastasis. A series of distinct molecular events appears to be involved in triggering EMT, such as the activation of certain transcriptional pathways, alterations in the expression of surface molecules and changes in cytoskeletal proteins.⁶ Moreover, cytokines and bacterial products have been highlighted as EMT-predisposing factors.⁷

The loss of cell–cell adhesion, and the downregulation of E-cadherin and other epithelial markers (occludin, laminin-1, matrix metalloproteinase-9 (MMP-9), and integrin) are the principal characteristics of EMT. These changes can occur in concert with the upregulation of key transcription factors and the expression of associated mesenchymal markers, such as vimentin, N-cadherin, fibronectin, and laminin-5.⁸

This study aimed to compare the expression of transforming growth factor-beta 1 (TGF- β 1), vimentin and E-cadherin in the gingival tissue samples obtained from periodontally diseased patients and in the tissue samples taken from individuals with healthy gingiva.

Material and methods

Sample size

Based on a previous study by Arora et al., which stated that the expression of E-cadherin among controls was 99% and the expression for periodontitis was 44%, using a power of 80% and a significance level of 5%,⁹ 9 samples in each group were considered sufficient. The sample size was calculated using the PS: Power and Sample Size Calculation software, v. 3.1.2 (Vanderbilt University, Nashville, USA; <http://ps-power-and-sample-size-calculation.freedownloadscenter.com/windows/>).

Subjects and sample collection

This study was conducted according to the regulations of the Ethics Committee of the Faculty of Dentistry at Cairo University in Egypt. The research was approved by the institutional ethics committee, and written informed consent was obtained from all of the participants involved in the study. A total of 36 participants were recruited.

The participants were divided according to their periodontal status into 2 main groups: the control group ($n = 9$); and the periodontitis group ($n = 27$), which consisted of 3 subgroups (mild, moderate and severe periodontitis) with 9 patients in each subgroup. The patients were classified into the mild, moderate and severe periodontitis subgroups based on the collected clinical data, according to the American Academy of Periodontology criteria from 2015.¹⁰

The study was performed on the gingival tissue samples obtained from both chronic periodontitis patients and healthy individuals. All samples were kindly provided by donors from the outpatient clinics at the Faculty of Dentistry, Cairo University. The inclusion criteria for the healthy group were as follows: the absence of any clinical signs of gingival inflammation; probing depth (PD) ≤ 3 mm; and no clinical attachment loss (CAL). The inclusion criteria for the periodontitis group comprised a generalized presence of clinical signs of gingival inflammation, generalized PD ≥ 4 mm and generalized CAL ≥ 2 mm. Generalized chronic periodontitis is defined as periodontitis without a clear pattern of disease distribution in the affected teeth, with more than 30% of the teeth affected.¹¹ The exclusion criteria were the following: patients with any systemic diseases; smokers; pregnant or lactating women; patients with cervical/proximal/subgingival caries or restorations; and patients who received periodontal or antimicrobial therapy within 3 months before sampling. The gingival tissue samples were harvested from November 2017 until February 2018, during periodontal surgery and the extraction of periodontally hopeless teeth for the periodontitis patients, and during premolar extraction (orthodontic

treatment) or third molar extraction for the healthy control patients. Each specimen from each experimental group was divided into halves; one half was used for the light microscopic examination and the histomorphometric analysis, while the other half was used for the gene expression analysis.

To minimize the number of variables during the study, potential confounders were excluded within the exclusion and inclusion criteria. In addition, to avoid human variations and remove bias, the sample preparation, cutting and staining were completed in the same laboratory by the same technician, according to standardized criteria. Moreover, to avoid selection, performance and detection bias, the patients were defined eligible based on consecutive order. Blinding was applied for the assessors of both the histological and quantitative real-time polymerase chain reaction (RT-PCR) results.

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

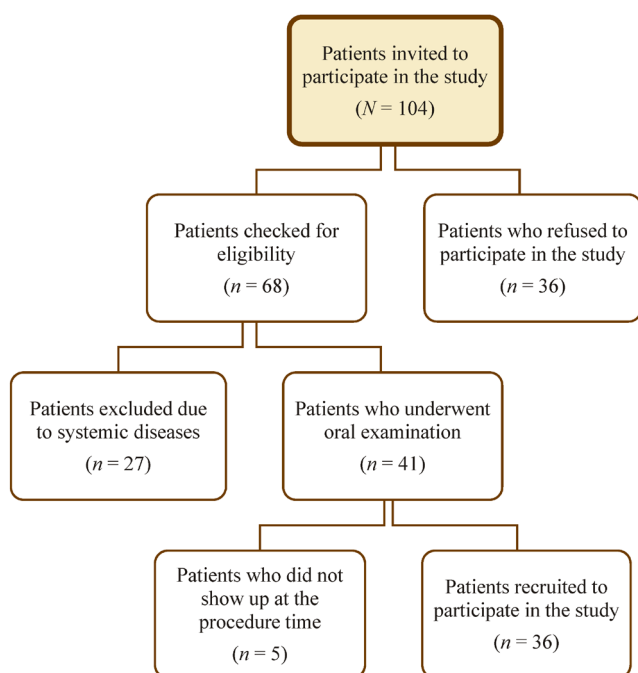


Fig. 1. Flow chart of the study

Experimental procedure

Light microscopic examination

The specimens were dehydrated in ascending grades of alcohol, then cleared in xylol and embedded in paraffin blocks. Sections of 4–5-micrometer thickness were mounted on ordinary glass slides and stained with hematoxylin and eosin (H&E) (Abbey Color, Philadelphia, USA) for routine histological evaluation, according to the conventional method.¹²

Histomorphometric analysis

The image analysis was performed using a Leica microscope equipped with a digital video camera and software (Leica Qwin 500; Leica Microsystems, Wetzlar, Germany). The image analysis system was used to assess the epithelial thickness and to count the number of inflammatory cells per area unit in order to assess the degree of inflammation.

Quantitative RT-PCR

The specimens were used to measure the expression of TGF- β 1, vimentin and E-cadherin. Total RNA was isolated using a tissue extraction kit (Qiagen Sciences Inc., Germantown, USA) according to the manufacturer's instructions. Total RNA (0.5–2 μ g) was used for cDNA conversion with a high-capacity cDNA reverse transcription kit (Fermentas, Waltham, USA). The quantitative RT-PCR amplification and analysis were performed using the StepOne™ system (Applied Biosystems, Waltham, USA). Relative quantification (RQ) was calculated using the Applied Biosystems software, v. 3.1 (Table 1).

Table 1. Primer sequences of the genes used in the study

Protein name	Gene symbol	Primer sequence (5'–3')
TGF- β 1	<i>TGF-β1</i>	forward – GCAGCACGTGGAGCTGTA reverse – CAGCCGGTTGCTGAGGTA
Vimentin	<i>VIM</i>	forward – CCCTCGCTCTCTTCTTGACAG reverse – AATGACTGCAGGGTGCTCTC
E-cadherin	<i>CDH1</i>	forward – TTCTCCGCTCCTGCTCTCT reverse – TTGTCAGTCTCTGGGCCGGT

TGF- β 1 – transforming growth factor-beta 1.

Reagents and equipment

In the present study, the following reagents and equipment were used:

- Moloney murine leukemia virus reverse transcriptase (M-MLV RT) for the synthesis of cDNA from RNA;
- human placental ribonuclease inhibitor;
- first-strand buffer;
- deoxynucleotide triphosphates (dNTPs) – dATP, dTTP, dGTP, and dCTP;
- random hexamers – primers for the reverse transcription of RNA (Stratagene California, La Jolla, USA);
- diethyl pyrocarbonate (DEPC)-treated water; and
- a thermal cycler (Biometra®; Analytik Jena, Jena, Germany).

Calculation of relative quantification (relative expression)

Relative quantification was calculated according to the Applied Biosystems software using the following formula (Equation 1):

$$RQ = 2^{-\Delta\Delta Ct} \quad (1)$$

where:

ΔCt = Ct gene test – Ct endogenous control;

$\Delta\Delta Ct$ = ΔCt treated sample – ΔCt calibrator;

Ct – cycle threshold.

Relative quantification is a fold change as compared to the calibrator (untreated sample).

Statistical analysis

The data is presented as means (M) \pm standard deviations (SD). The data was first tested for normality with the Kolmogorov–Smirnov test, which revealed that most data was parametric. The one-way analysis of variance (ANOVA) was used for comparisons between the groups. This was followed by Tukey's post hoc test when ANOVA revealed a significant difference. The level of significance was established at $p < 0.05$.

The analysis of correlation between various variables was performed using the Pearson product–moment correlation equation for the linear relation of normally distributed variables. Two-sided $p < 0.05$ was considered statistically significant. All statistical calculations were performed with the use of the IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA).

The power analysis was performed for the only statistically insignificant variable. The mean value of the variable in each group was entered and the highest SD was selected as an intragroup variation parameter. The effect size was calculated, and then entered to calculate the power of the statistical results. The omnibus one-way ANOVA test was used in the analysis with a type I error probability equal to 0.05. The calculations were performed using the G*Power software v. 3.1.2 for Windows (<https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower>).

Results

Light microscopic examination

Epithelium

The gingival epithelium of the moderate and severe periodontitis subgroups showed epithelial hyperplasia, while that of the mild periodontitis subgroup exhibited

a mild increase in the epithelial thickness as compared to the control group (Fig. 2). The epithelial ridges of the moderate and severe periodontitis subgroups showed elongation and a tortuous appearance. When examining the mild periodontitis subgroup, however, it was observed that the epithelial ridges had a configuration similar to that of the control group. The basement membrane was intact in both the control group and the mild periodontitis subgroup, while there was a loss of continuity in some areas in the moderate and severe periodontitis subgroups (Fig. 2).

The moderate and severe periodontitis subgroups showed migrating inflammatory cells that were scattered throughout the layers of the epithelium, but were mainly concentrated in the basal and parabasal cell layers. On the other hand, there were only a few of these cells in the epithelium of the mild periodontitis subgroup and the control group (Fig. 3).

Perinuclear haloing was observed in the basal and parabasal cell layers in both the moderate and severe periodontitis subgroups. Conversely, no similar haloing was observed in the basal and parabasal cell layers in the mild periodontitis subgroup and the control group (Fig. 3). Severe perinuclear haloing was observed in the prickle cell layers in the tissue samples from the moderate and severe periodontitis patients (Fig. 3). Haloing was also noted in the prickle cell layers in the samples from the mild periodontitis subgroup and the control group, but to a lesser extent (Fig. 4).

The flattening of the granular cell layer and the disappearance of its keratohyalin granules were detected in the gingiva from the severe periodontitis patients. The

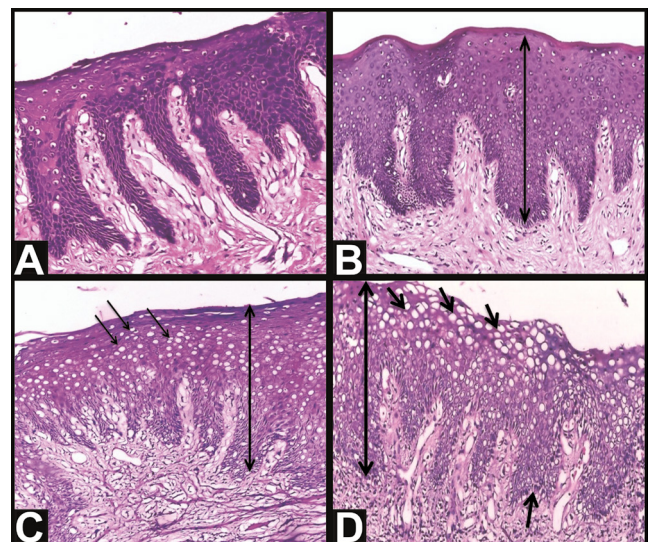


Fig. 2. Photomicrographs of the gingiva from the control group (A) and the mild periodontitis subgroup (B), showing the mild elongation (double-head arrow) and normal configuration of the epithelial ridges, and of the moderate (C) and severe (D) periodontitis subgroups, showing epithelial hyperplasia, and the elongation (double-head arrows) and tortuous appearance of the epithelial ridges (single-head arrows)

Hematoxylin and eosin (H&E) stain; $\times 40$ magnification.

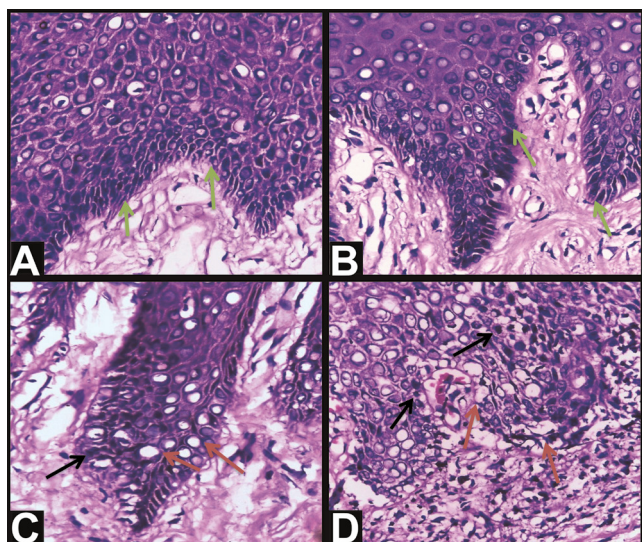


Fig. 3. Photomicrographs of the gingiva from the control group (A) and the mild periodontitis subgroup (B), showing the basal and parabasal cell layers with the absence of perinuclear haloing and an intact basement membrane (green arrows), and of the moderate (C) and severe (D) periodontitis subgroups, showing migrating inflammatory cells (black arrows), perinuclear haloing in the basal, parabasal and prickle cell layers, and the loss of continuity of the basement membrane (red arrows) H&E stain; $\times 40$ magnification.

gingiva from the moderate periodontitis subgroup also showed the disappearance of keratohyalin granules and perinuclear haloing in the granular cells. On the other hand, keratohyalin granules were present in the gingival tissues of the mild periodontitis subgroup and the control group (Fig. 4).

The gingival epithelium from the moderate and severe periodontitis subgroups showed a reduction of the keratin layer. There was also severe vacuolization,

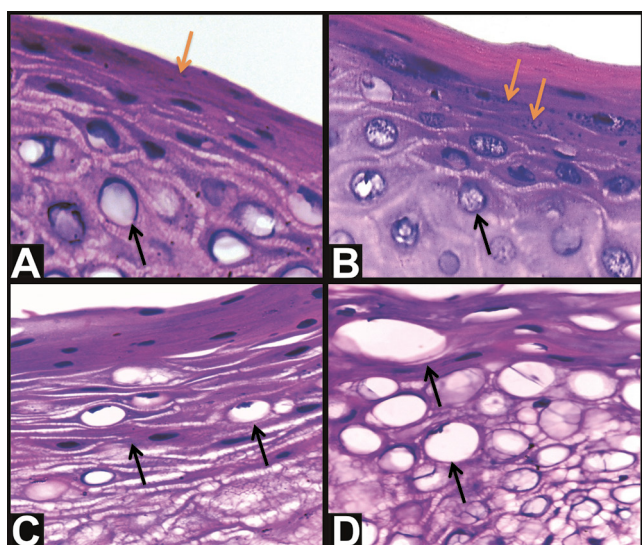


Fig. 4. Photomicrographs of the gingiva from the control group (A), and the mild (B), moderate (C) and severe (D) periodontitis subgroups, showing changes in the keratin layer and perinuclear haloing in the superficial and prickle cell layers (black arrows) with a gradual loss of keratohyalin granules (orange arrows)

H&E stain; $\times 100$ magnification.

and perinuclear haloing was present in the superficial cells. Conversely, the gingiva from the mild periodontitis subgroup showed that keratin was present, which was similar to what was observed in the control group (Fig. 4).

Lamina propria

The gingival tissues from all of the periodontitis patients showed inflammatory cell infiltration in their papillary and reticular layers at different grades, in proportion to the severity of periodontal inflammation. On the contrary, the samples from the control group showed very few inflammatory cells (Fig. 5).

The mild periodontitis subgroup showed few capillaries and dilated small blood vessels engorged with blood. In the moderate periodontitis subgroup there was a slight increase in the number of small capillaries and blood vessels, while the severe periodontitis subgroup showed a marked increase in vasculature (the formation of small capillaries) and few moderate-sized blood vessels (Fig. 5).

Histomorphometric analysis

The highest mean epithelial thickness was recorded in the severe periodontitis subgroup ($178.348 \mu\text{m}$), whereas the lowest mean value was recorded in the control group ($89.778 \mu\text{m}$). The difference between the groups was statistically significant ($p < 0.028$). There were no significant differences between the control group and the mild and moderate periodontitis subgroups ($p > 0.05$) (Table 2).

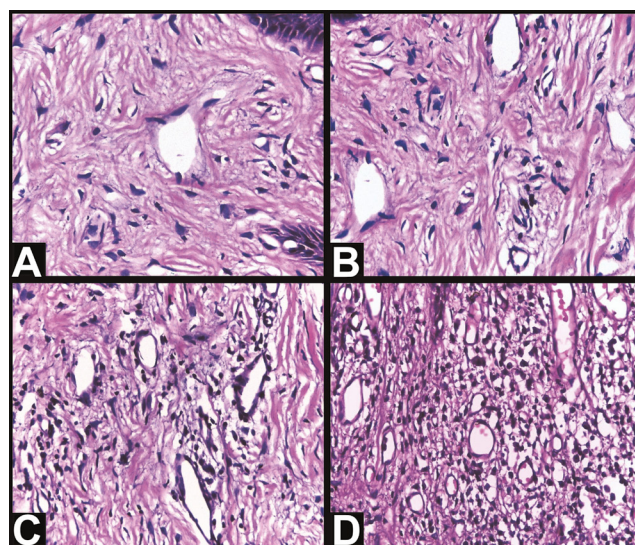


Fig. 5. Light micrographs of the connective tissue from the control group (A), and the mild (B), moderate (C) and severe (D) periodontitis subgroups, showing differences in collagen fiber orientation, and a gradual increase in the blood vessel size and the number of inflammatory cells

H&E stain; $\times 40$ magnification.

Table 2. Descriptive statistics regarding the epithelial thickness and the inflammatory cell count, and comparisons between the control group and the periodontitis subgroups (ANOVA)

Measured items	Groups	<i>M</i> ± <i>SD</i>	<i>p</i> -value
Epithelial thickness [μm]	control group	89.778 ^b ± 20.546	<0.028*
	mild periodontitis subgroup	101.391 ^{ab} ± 37.607	
	moderate periodontitis subgroup	145.763 ^{ab} ± 50.985	
	severe periodontitis subgroup	178.348 ^a ± 63.643	
Inflammatory cell count [cells/μL]	control group	44,523 ± 25,993	<0.177
	mild periodontitis subgroup	74,865 ± 43,791	
	moderate periodontitis subgroup	95,414 ± 61,621	
	severe periodontitis subgroup	123,313 ± 74,124	

M – mean; *SD* – standard deviation; * statistically significant ($p < 0.05$); means sharing the same superscript letter within the same comparison are not significantly different.

The highest mean count of inflammatory cells was recorded in the severe periodontitis subgroup (123,313 cells/μL), whereas the lowest mean value was recorded in the control group (44,523 cells/μL). The ANOVA test revealed that the difference between the groups was not statistically significant ($p < 0.177$) (Table 2).

The power analysis that was carried out for the statistically insignificant variable (inflammatory cell count) revealed that the calculated effect size was 0.389 and the calculated power was 42.7%.

Quantitative RT-PCR

The highest mean TGF-β and vimentin expression values were recorded in the severe periodontitis subgroup (559.60 and 227.60, respectively), whereas the lowest mean values were recorded in the control group (48.84 and 32.18, respectively). The difference between

these 2 groups was statistically significant ($p < 0.001$). On the contrary, there was no significant difference between the control group and the mild periodontitis subgroup (Table 3).

The highest mean E-catherin expression value was recorded in the control group, whereas the lowest mean value was recorded in the severe periodontitis subgroup. The difference between these 2 groups was statistically significant ($p < 0.001$). Moreover, there were statistically significant differences between all the studied groups (Table 3).

The correlation analysis between the severity of periodontitis and the expression of both TGF-β1 and vimentin revealed a statistically significant positive correlation ($r = 0.895$; $p < 0.001$ and $r = 0.857$; $p < 0.001$, respectively), while E-catherin was found to be significantly negatively correlated ($r = -0.976$; $p < 0.001$) (Table 3).

Table 3. Descriptive statistics regarding the quantitative real-time polymerase chain reaction (RT-PCR) analysis of the expression of TGF-β, vimentin and E-cadherin, comparisons between the control group and the periodontitis subgroups (ANOVA), and the correlation analysis (Pearson correlation coefficient (*r*))

Measured items	Groups	<i>M</i> ± <i>SD</i>	95% <i>CI</i>		<i>p</i> -value (ANOVA)	Correlation	
			lower bound	upper bound		<i>r</i>	<i>p</i> -value
TGF-β1	control group	48.84 ^c ± 3.02	45.09	52.59	<0.001*	0.895	<0.001*
	mild periodontitis subgroup	99.50 ^c ± 25.33	68.05	130.95			
	moderate periodontitis subgroup	299.20 ^b ± 47.39	240.35	358.05			
	severe periodontitis subgroup	559.60 ^a ± 211.51	296.97	822.23			
Vimentin	control group	32.18 ^b ± 3.89	27.35	37.01	<0.001*	0.857	<0.001*
	mild periodontitis subgroup	45.50 ^b ± 6.03	38.01	52.99			
	moderate periodontitis subgroup	105.32 ^b ± 36.43	60.09	150.55			
	severe periodontitis subgroup	227.60 ^a ± 76.73	132.32	322.88			
E-cadherin	control group	7.00 ^d ± 0.16	6.80	7.20	<0.001*	-0.976	<0.001*
	mild periodontitis subgroup	5.80 ^c ± 0.56	5.11	6.49			
	moderate periodontitis subgroup	3.06 ^b ± 0.85	2.01	4.12			
	severe periodontitis subgroup	1.09 ^a ± 0.25	0.78	1.39			

CI – confidence interval; * statistically significant ($p < 0.05$); means sharing the same superscript letter within the same comparison are not significantly different.

Discussion

The purpose of this study was to localize and quantify TGF- β 1 (a cytokine), vimentin (an intermediate filament) and E-cadherin (a cell adhesion molecule) related to the EMT process in human gingival tissue samples from periodontally diseased patients, and to compare them to samples from healthy individuals.

A gradual increase in the thickness of the epithelium was detected in the mild, moderate and severe periodontitis subgroups. The histomorphometric analysis supported this finding, as the highest mean epithelial thickness was recorded in the severe periodontitis gingival tissues, while the lowest was recorded in the control group. This difference was statistically significant ($p < 0.028$).

These findings are in agreement with Tanaskovic Stankovic et al., who reported that an increase in the gingival epithelium was proportional to the severity of inflammation.¹³ The thickening in the inflamed gingival epithelial tissues in the present study could be attributed to a disturbance in tissue homeostasis and the upregulation of inflammatory cytokines, such as interleukin (IL)-1, IL-3, IL-6, and IL-8, which have proliferative effects on human keratinocytes in vitro.¹⁴

This study reported a reduction in keratinization, especially in the moderate and severe periodontitis subgroups. These findings support the hypothesis that there is a direct relationship between the degree of inflammation and a reduction in keratinization.¹⁵ In both the moderate and severe periodontitis subgroups, the granular cell layer was flattened and transformed into a superficial cell layer as a means of mechanical protection to compensate for the decreased keratin layer thickness.

The absence of keratohyalin granules observed in the moderate and severe periodontitis subgroups might explain the decrease or absence of keratin detected in both groups. This is because keratohyalin granules play an important role in the synthesis of keratin in keratinized or parakeratinized stratified squamous epithelium.¹⁶ This point is further supported in this study by the presence of these granules associated with keratin in the gingival specimens of both the mild periodontitis subgroup and the control group.

The severe perinuclear haloing detected throughout the epithelial cell layers in both the moderate and severe periodontitis subgroups is consistent with the findings of Abbass et al.; they reported perinuclear haloing in periodontitis patients when compared with healthy gingiva.¹⁷ This haloing could be attributed to apoptotic changes that occurred in these cells and the immune system response against severe inflammation. This is supported by the increase in TGF- β 1 expression in the periodontitis subgroups reported in the present study. Perinuclear haloing was also detected in both the mild periodontitis and healthy tissue samples, but to a lesser degree, as the severity of inflammation diminished.

In the present investigation, the basement membrane of the moderate and severe periodontitis subgroups showed the loss of continuity in some areas, while the basement membrane had a normal appearance in the mild periodontitis subgroup and the normal tissue group. These results are consistent with the findings of Kantarci et al., who reported basement membrane discontinuities in overgrown tissues due to periodontal inflammation when compared to control tissues.¹⁸ Moreover, these findings clarify the role played by EMT in the pathogenesis of periodontitis, as the loss of basement membrane integrity makes it easier for bacterial pathogens to invade the underlying tissues.

The histomorphometric analysis regarding inflammatory cells determined that the highest mean value was observed in the severe periodontitis subgroup, whereas the lowest mean value was in the control group; however, this difference was not statistically significant ($p < 0.177$). These findings are in agreement with Zekonis et al., who reported higher values for lymphocytes and macrophages in patients with chronic periodontitis than in healthy individuals.¹⁹ Castro et al. also reported a significant gradual increase in the number of lymphocytes in gingival tissue samples, coinciding with the severity of the disease in chronic periodontitis patients.¹⁵

The lamina propria in the periodontitis subgroups showed a direct increase in the numbers of blood vessels, correlated with the severity of periodontal disease. An increase in the amount of new vasculature is a common sign of inflammation that is known to be mediated by different inflammatory cytokines, including TGF- β 1, interleukins and others.²⁰ These cytokines have been shown to be involved in the initiation of the EMT process.²¹ The data confirms that there is a correlation between the incidence of EMT and the pathogenesis of inflammatory periodontal disease. This is consistent with the results from a previous study that reported a significant increase in the number of blood vessels in the lamina propria of adult chronic periodontitis specimens when compared to healthy tissues.²²

The current study reported a significant gradual increase in TGF- β 1 expression in the mild, moderate and severe periodontitis gingival tissues. These results indicate that there is a direct relationship between the severity of inflammation and the expression of TGF- β 1 in tissues, which in turn represents the activity of the proposed EMT process. These findings are consistent with those of Mize et al., who deduced that TGF- β 1 mRNA expression levels were significantly increased in individuals with periodontitis when compared to individuals without periodontitis.²³ Matarese et al. attributed high TGF- β 1 levels in their periodontitis patients to the body's protective response against periodontitis.²⁴ Potentially, it may be that the biological activities of these growth factors resulted in the insufficient remodeling and perfusion of tooth-supporting tissues, and contributed to periodontal destruction.

A significant decrease in the transcription of E-cadherin in the mild, moderate and severe periodontitis tissue samples was demonstrated in this study. The downregulation of E-cadherin supports the incidence of EMT during periodontitis, as the epithelial cells lose their cellular junctions. These findings are in agreement with the results from a previous study, which demonstrated the downregulation of E-cadherin expression in the inflamed periodontal tissue in vivo.²⁵ Moreover, Arun et al. reported that the virulence factors produced by *Porphyromonas gingivalis* were capable of decreasing epithelial junctional attachments primarily through the downregulation of E-cadherin.²⁶ The authors noted that treating periodontal pockets resulted in a significant upregulation of E-cadherin expression in comparison with diseased sites.²⁶

The inverse relationship between E-cadherin and TGF- β 1 expression detected in the current study is consistent with the findings of Cho et al., who reported in 2010 that E-cadherin antagonized the induction of the TGF- β 1 gene in hepatic cells.²⁷

The present study reported a significant upregulation in vimentin expression in all 3 periodontitis subgroups. The RT-PCR statistical analysis showed that the highest mean value was recorded in the severe periodontitis subgroup, whereas the lowest mean value was recorded in the control group; this difference was statistically significant ($p < 0.001$). These results in addition to the E-cadherin results confirmed that EMT occurs during periodontitis, as the epithelial cells lose their characteristic features and acquire a mesenchymal phenotype. In agreement with these findings, an in vitro study reported that the exposure of H400 keratinocytes to bacterial components revealed the upregulation of vimentin expression.²⁸ Also, Scanlon et al. reported a simultaneous upregulation of vimentin expression after stimulation with heat-killed periodontal pathogens and *Escherichia coli* lipopolysaccharides.²⁹ Similar results were obtained in another study, in which 4 epithelial cell lines (hepatocellular carcinoma cell lines) were exposed to lipopolysaccharides.³⁰

In the current study, the high expression of vimentin recorded in the periodontitis subgroups could be related to the high expression of TGF- β 1 also observed in these subgroups. The correlation between TGF- β 1 and vimentin was investigated by Yoshida et al., who reported that treating mammary epithelial cells with TGF- β 1 induced the expression of vimentin, which was associated with increased cell invasiveness.³¹ Therefore, it could be concluded that there is a direct positive correlation between TGF- β 1 and vimentin.

The strategies used for treating periodontitis have not changed much over the last decades. Most of these strategies focus on reducing the plaque biofilm.³² In the case of aggressive periodontal disease, antibiotic regimens are used to aid in the elimination of bacterial pathogens.³³


These approaches do not always provide successful outcomes, and are responsible for local and systemic side effects. Furthermore, there is increased concern about bacterial resistance. A further issue with periodontal disease management is the decolonization of the treated periodontal lesion from bacterial reservoirs within the oral cavity.³⁴


To date, few clinical trials have investigated interventions that aim to modulate the host response. Moreover, there is scarce experimental research investigating naturally derived anti-EMT treatment.³⁵ The data presented in the current study could provide a potential justification for using anti-EMT agents in managing periodontal disease. Maintaining a functional and intact epithelial barrier would prevent the bacterial invasion of the connective tissues and the subsequent aberrant stimulation of the host response, which is involved in tissue breakdown. Ultimately, the question that needs to be addressed before the potential use of EMT blockers in periodontal disease management is whether EMT is an integral part of the cellular survival mechanism or is a failed component of the healing process.


Conclusions

The data in this study could potentially provide a basis for using anti-EMT agents in managing periodontal disease, since a significant direct correlation between the severity of periodontal disease and the expression of EMT markers was proven.

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Prospective evaluation of vitamin D levels in dental treated patients: A screening study

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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 available epidemiological data indicates the existence of numerous tooth deficiencies and periodontopathies in the Polish population. Successful surgical treatment is dependent upon multiple factors, including bone quality, which is affected by vitamin D – one of the regulators of calcium (Ca) and phosphorus (P) metabolism.

Objectives. The aim of this study was to conduct a prospective evaluation of the levels of vitamin D among patients receiving dental treatment for various reasons (conservative, surgical or prosthodontic treatment).

Material and methods. The study involved 60 patients, who, for various reasons, were admitted for dental treatment for over a 1-week period in the summer season. A thorough interview together with a periodontological examination consisting of probing depth (PD) and clinical attachment level (CAL) measurements, were conducted. A total of 2 mL of blood was collected from each patient to determine the concentrations of vitamin D, parathyroid hormone (PTH), Ca, magnesium (Mg), and P in the blood with the use of mass spectrometry.

Results. Among the 60 patients enrolled in the study, 53 (88.3%) showed vitamin D deficiency and 18 (30%) showed a concentration below 17.8 ng/mL, which is an independent general mortality factor. The difference in the level of vitamin D between group A (without periodontitis) and group B (with periodontitis) was not statistically significant ($p = 0.076$), but a tendency for lower vitamin D levels in people with periodontitis was observed. We also observed a tendency for periodontal disease to occur more often with extreme vitamin D deficiency (< 15 ng/mL) ($p = 0.730$).

Conclusions. Low levels of vitamin D are present in a large proportion of dental patients, and an increase in the incidence of periodontal disease is likely associated with a decrease in the level of vitamin D.

Keywords: vitamin D, calcitriol, dental treatment, vitamin D deficit

Cite as

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Introduction

Research to date has indicated that periodontopathies are socially debilitating^{1–6} and affect ~90% of the adult Polish population.^{7–8} Indeed, an average Polish person over 35 has only 21 of their own teeth. This data shows the scale of periodontal disease in Poland, and indicates the need for periodontal and implant treatment in these patients.

Vitamin D occurs in 2 main forms: cholecalciferol (vitamin D3 of animal origin) and ergocalciferol (vitamin D2 of plant origin). This vitamin is a derivative of cholesterol and belongs to the family of secosteroid compounds. Vitamin D can be obtained from a proper diet; however, the vast majority (~90%) is synthesized in the human organism. The synthesis of the active form of vitamin D (calcitriol) is accomplished in several stages. The precursor, 7-dehydrocholesterol, can be found in the cell membranes of keratinocytes in the basal and spinous layers (collectively known as the Malpighian layer), and in the fibroblasts of the dermis. Upon exposure to ultraviolet (UV) radiation (290–315 nm), 7-dehydrocholesterol is converted into lumisterol and tachysterol. Then, under the influence of heat (skin temperature of ~25°C) and the reductase enzyme, these compounds are isomerized to cholecalciferol (calcio). Cholecalciferol is then released into the blood, where it binds to a specific vitamin D-binding globulin (protein DBP) and is transported to the liver. In the liver, hydroxylation occurs via 25-hydroxylase, leading to the formation of 25-hydroxy-cholecalciferol (25(OH)D; calcidiol). The biological activity of this compound is low and its excess is stored in adipocytes. The next stage takes place in the proximal tubules of the kidneys, where 1 α -hydroxylase (CYP27B1) converts calcidiol into 1,25-dihydroxycholecalciferol (1,25(OH)2D; calcitriol). Known as the vitamin hormone, the activity of calcitriol is 10,000 times higher than that of calcidiol, but its half-life is shorter, lasting 4–6 h. The 1 α -hydroxylation process also takes place in other cells and organs, such as macrophages, keratinocytes, the placenta, the parathyroid glands, prostate cells, osteoblasts, immune system cells, pancreatic islands, vascular smooth muscles, and cancer cells, where, through auto- and paracrine regulation, locally synthesized vitamin D affects local physiological processes.^{9–11}

Chronic vitamin D deficiency results in abnormal calcium (Ca) absorption in the small intestine, which can lead to a reduction in Ca blood concentration. Under these circumstances, the parathyroid glands increase the production of parathyroid hormone (PTH), which, by affecting the bone, increases the release of Ca into the blood to compensate for the deficiency. This compensatory process is driven by the activation of osteoclasts, which can result in the destruction of bone tissue, leading, in turn, to a reduction in bone calcification, rickets, osteomalacia, and osteoporosis. The role of vitamin D in the bone mineralization process is further supported by the observation that bone tissue deteriorates during the

winter/spring, when the synthesis of vitamin D through the action of sunlight is considerably reduced. Vitamin D can reduce bone resorption by increasing the intestinal absorption of Ca and its reabsorption in the distal renal tubules. However, it also accelerates the secretion of calcitonin and decreases the proliferation of parathyroid cells, which, in turn, inhibits the synthesis and activity of PTH. Vitamin D also aids bone repair processes and bone matrix synthesis, reduces the perforation of trabeculae, and increases the activity of bone growth factors.

Objectives

The aim of the paper was to conduct a prospective, observational screening study of the levels of vitamin D and PTH in patients undergoing dental treatment for various reasons.

Material and methods

This research was performed in full compliance with the Declaration of Helsinki and was approved by the local ethics committee (229/2019).

The study included patients who were, for various reasons, admitted to a private dental clinic for treatment for over a 1-week period during the summer season. The patients were thoroughly interviewed to obtain information concerning their general condition, and examined intraorally with a focus on history of chronic periodontal disease, tobacco smoking and the need for implant treatment. The patients were also informed about the importance of determining the level of vitamin D before each kind of dental treatment and its impact on general health. The study involved patients over 18 of both sexes. The exclusion criteria comprised active vitamin D supplementation, the use of UV lamps, pregnancy, and breastfeeding. Patients with general diseases that affect the level of vitamin D, such as obesity, type 1 diabetes mellitus, inflammatory bowel disease, bronchial asthma, celiac disease, or hypertension, were also excluded from the study. All patients gave their voluntary and written consent to participate.

The incidence of periodontal disease was assessed in accordance with the classification of the American Academy of Periodontology for the Centers for Disease Control and Prevention (CDC); however, without the application of the disease severity classification.¹² Probing depth (PD) from the margin of the gingiva to the bottom of the pocket was measured with a probe (in millimeters) on 4 surfaces of the following teeth: 16; 11; 26; 36; 31; and 46 (right upper first molar; right upper medial incisor; left upper first molar; left lower first molar; left lower medial incisor; and right lower first molar). If the patient was lacking any of the examined teeth, the examination was not conducted at the site of the deficiency. If 2 or more sites with a periodontal pocket of at least 4 mm were ob-

served, it was marked as periodontitis. The distance from the enamel–cement margin to the bottom of the pocket, measured with the use of a probe in millimeters on 4 surfaces of teeth 16, 11, 26, 36, 31, and 46, constituted the clinical attachment level (CAL).

A total of 2 mL of blood was collected from each patient's ulnar pit in order to determine the levels of vitamin D, PTH and electrolytes (Ca, magnesium (Mg) and phosphorus (P)). After collection, the samples were set aside for 20 min to coagulate at room temperature, and then were centrifuged at 3,000 rpm for 10 min. The serum was transferred to glass tubes marked with the code of the patient and subsequently frozen at -20°C . The samples were sent for analysis to the Laboratory of Quantitative Analysis of Drugs and Metabolites, which is part of the Laboratory of Mass Spectrometry in the Institute of Biochemistry and Biophysics at the Polish Academy of Sciences in Warsaw.

Vitamin D concentration in the blood serum was determined by means of liquid chromatography with tandem mass spectrometry (LC-MS/MS). This method is based on the technique of distinguishing single ions of the substances in the sample, using the measurements of the ion mass to ion charge ratio. Due to the stability of 25(OH) vitamin D in the blood serum, and the fact that the rate of its synthesis depends on the amount of vitamin D supplied with food, medications or supplements, it is believed to be the best indicator of vitamin D concentration in the organism. Laboratory methods measuring both the 25(OH)D2 and the 25(OH)D3 subunit, the sum of which constitutes the total concentration of vitamin D in the serum, were applied.

Statistical analysis was carried out using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA).

Results

Considering the results, it is worth noting that the level of vitamin D was measured in the summer, in August, which is associated with an increased vitamin D synthesis in the skin due to increased exposure to sunlight. Currently, a vitamin D level below 30 ng/mL is considered to be deficiency, while extreme deficiency is diagnosed when the value is below 15 ng/mL. The reference ranges for PTH, Ca, Mg, and P are 15.065 pg/mL, 8.8–10.2 mg/dL, 1.58–2.55 mg/dL, and 2.7–4.5 mg/dL, respectively.

Sixty patients were enrolled in the study, including 42 women (70%) and 18 men (30%). The overall mean age of the patients was 46 years (min 20, max 85), and the overall mean concentration of vitamin D was 23.2 ng/mL. For women, the mean age was 44 and for men the mean age was 57, and the mean levels of vitamin D for these groups were 24.28 ng/mL and 21.89 ng/mL, respectively ($p > 0.05$; Mann–Whitney U test).

A decreased level of vitamin D was observed in 53 individuals (88.3% of the group). These patients had a mean age of 47 years and an average vitamin D concentration of 21.7 ng/mL, without any predilection with regard to sex. Among this group of patients, 37 were women (mean age 44) with a vitamin D concentration of 20.75 ng/mL, while 16 were men (mean age 56) with a mean value of vitamin D concentration of 20.63 ng/mL. Among patients with a normal concentration of vitamin D, the average value was only 37 ng/mL. In as many as 13 individuals, including 11 women and 2 men, extreme vitamin D deficiency (<15 ng/mL) was observed, with an average of 12 ng/mL.

To examine the potential associations between periodontal disease and vitamin D levels, the patients were divided into 2 groups. The 1st group (A) consisted of 28 patients without periodontal disease and the 2nd group (B) comprised 32 patients with periodontal disease (53.3% of all patients; mean age 55). The mean vitamin D levels were 24.8 ng/mL in group A and 21.7 ng/mL in group B. There was no statistically significant difference between these groups ($p = 0.076$; Mann–Whitney U test). In 30 persons from group B, the level of vitamin D was decreased (mean (M): 19.2 ng/mL; mean age 56).

It is noticeable that the incidence of periodontal disease increases with a decrease in the level of vitamin D. Three people out of 7 (42.9%) had periodontal disease in the group where the concentration was normal. In the group with vitamin D levels at the range of 15–30 ng/mL, 21 people out of 40 (52.5%) showed periodontal disease, and in the group with vitamin D levels <15 ng/mL, it was 8 people out of 13 (61.5%) (Fig. 1). We could also observe a tendency for periodontal disease to occur more often in patients with extreme vitamin D deficiency (<15 ng/mL) as compared to those with a normal vitamin D concentration (>30 ng/mL) ($p = 0.730$; χ^2 test).

It was noticed that the level of vitamin D decreased with age. The mean age when the level of vitamin D was <15 ng/mL was 51, and for >30 ng/mL, it was 47. However, this difference was not statistically significant ($p > 0.05$; Mann–Whitney U test).

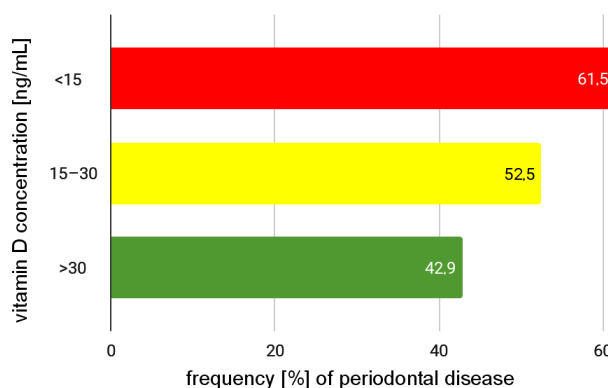


Fig. 1. Distribution of the incidence of periodontal disease in relation to vitamin D concentration

Data presented as means (M).

The average concentration of vitamin D in women above 50 was 22.15 ng/mL, which can be associated with an increased risk of osteoporosis and a decrease in estrogen concentration.¹³

It was also observed that the concentration of PTH increased with a decrease in the level of vitamin D (>30 ng/mL, 15–30 ng/mL, <15 ng/mL), but there were no statistically significant differences between these groups ($p > 0.05$; Mann–Whitney U test). In individuals with a normal concentration of vitamin D, the mean level of PTH was 34.56 pg/mL, in those with a concentration of vitamin D <30 ng/mL, the mean level of PTH was 43.84 pg/mL, and in the case of extreme vitamin D deficiency, the mean level of PTH was 47.54 pg/mL. Overall, the average level of PTH was 44.37 pg/mL. In group B (with periodontal diseases), the mean level of PTH was 48.65 pg/mL, and in group A (without periodontal diseases), the mean level was 38.75 pg/mL.

The concentrations of electrolytes (Ca, Mg and P) were within the normal range for all participants. When the concentration of vitamin D was 15–30 ng/mL, the concentration of Ca amounted to 9.40 mg/dL, the concentration of Mg was at 1.86 mg/dL and the concentration of P was 3.57 mg/dL. When the level of vitamin D was <15 ng/mL, the values for these electrolytes were equal to 8.81 mg/dL, 1.83 mg/dL and 3.31 mg/dL, respectively (Fig. 2). The overall average levels for these electrolytes were as follows: Ca – 9.47 mg/dL; Mg – 2.02 mg/dL; and P – 3.42 mg/dL. In group B (with periodontal disease), they were: Ca – 9.52 mg/dL; Mg – 1.98 mg/dL; and P – 3.32 mg/dL, and in group A (without periodontal disease), they were: Ca – 9.43 mg/dL; Mg – 2.03 mg/dL; and P – 3.52 mg/dL.

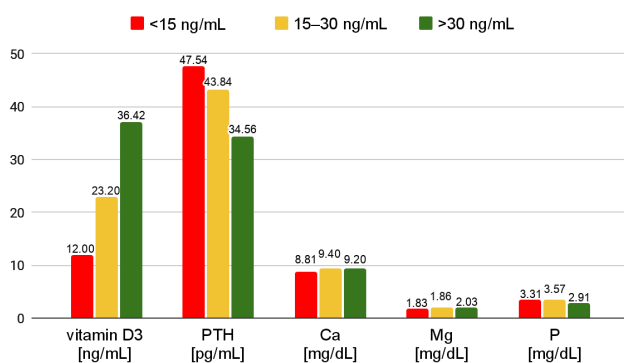


Fig. 2. Distribution of parameters depending on the study group

PTH – parathyroid hormone; Ca – calcium; Mg – magnesium; P – phosphorus; bars refer to groups with various levels of vitamin D. Data presented as means (M).

It should be emphasized that a vitamin D concentration below 17.8 ng/mL is considered to be an independent general mortality factor.¹⁴ In our study, such concentration or lower was observed in 18 patients, which constitutes 30% of all patients.

Discussion

Vitamin D plays an important role in the stomatognathic system, where its impact on caries prevention has been shown.¹⁵ This vitamin can also modulate Behçet's disease, aphthosis and Sjögren's syndrome.¹⁶ Vitamin D3 hypovitaminosis is a risk factor for the occurrence of pathological periodontal pockets and gingivitis, and the loss of bone mass; it can also decrease the efficacy of the organism's defense systems.¹⁷ This vitamin is capable of modifying the course of periodontal disease through 3 basic mechanisms: the maintenance of normal bone metabolism; antibacterial activity; and anti-inflammatory activity.¹⁸ The antibacterial activity of vitamin D is mainly based on the induction of the secretion of beta-defensins and cathelicidin LL-37. The anti-inflammatory activity is realized through the inhibition of the release of cytokines interleukin (IL)-1 β , IL-6 and IL-12, a decrease in the number of T and B cells, and blocking the pro-inflammatory activity of Th1 and Th17 cells. This activity of vitamin D was confirmed in a study conducted by Oteri et al., where it was demonstrated that an increase in the level of vitamin D reduced the inflammatory response and resulted in improved healing after the surgical extraction of third molar.¹⁹

In light of the abovementioned reports, scientists began to examine the possible correlations between the activity of vitamin D and the course of periodontal disease more carefully. There are reports that link vitamin D to a decreased loss of the alveolar process bone and a decreased loss of connective tissue attachment, while others have shown positive effects of vitamin D activity and Ca supplementation in terms of improved bone density.^{20–22} Bashutski et al. assessed the effects of vitamin D on wound healing and periodontal treatment results.²³ In patients with initial vitamin D deficiency, an increase in CAL was much smaller and PD was much greater than in individuals where the level of vitamin D was normal.²³ Leszczyszyn also observed that periodontal disease occurred much more frequently in patients who did not supplement vitamin D (59.3% vs 39.3%, respectively).²⁴ The percentage of patients with a low value of CAL (60.5% vs 32.0%; $p = 0.010$), recessions (59.3% vs 35.7%; $p = 0.030$), pathological alveolar pockets (33.7% vs 10.7%; $p = 0.028$), and periodontal disease (60.5% vs 35.7%; $p = 0.039$) was greater in the group of individuals with vitamin D deficiency as compared to the group of individuals where the concentration of vitamin D was optimal.²⁴

Studies examining the impact of vitamin D on implant treatment and bone augmentation procedures also can be found in the literature. Schulze-Späte et al. investigated whether oral supplementation with vitamin D3 (5,000 IU) in combination with Ca (600 mg) would affect bone formation and reconstruction after maxillary sinus augmentation; however, no statistically significant difference was observed in this study.²⁵ In some clinical situations, an alternative to regenerative procedures is the use of short dental implants. Studies have shown that, in many clinical situations, short

implants are a good alternative to the regular length of implants, despite the often unfavorable ratio of the crown length to the implant length.^{26,27} Studies on the impact of vitamin D deficiency on implant loss due to incorrect osseointegration have been carried out. A tendency (without statistical significance) for an increased risk of implant loss with an increase in vitamin D deficiency was demonstrated.^{28–30}

Given the above findings, it has become clear that it is necessary to increase the level of vitamin D through proper supplementation.³¹ The reasons for the observed deficiency may include the impairment of supply and intestinal absorption, sunlight deficiency, disturbed renal and/or hepatic metabolism, or disorders associated with vitamin D receptor (VDR) abnormalities.³² It is also extremely important to take vitamin K2MK7, as one of its functions includes the activation and enhancement of GLA proteins, including osteocalcin and matrix GLA protein (MGP), which can affect bone quality. Vitamin D should be supplemented in drops, taken after meals for better absorption.³³ The recommended total dose of vitamin D during the treatment can be calculated on the basis of Gronningen's formula (Equation 1):

$$40 \times (75 - \text{concentration of 25(OH)D [nmol/l]}) \times \text{body mass [kg]} \quad (1)$$

where:

$$1 \text{ ng/mL} = 2.5 \text{ nmol/mL}^{34}$$

Vitamin D can be supplied with the administration of ergocalciferol (vitamin D₂), cholecalciferol (vitamin D₃) or calcifediol (25(OH)D₃). The latter is used in the treatment of liver diseases, and for complications associated with glucocorticosteroid treatment or treatment with antiepileptic drugs. The administration of calcitriol and its derivatives has no impact on the improvement of vitamin D₃ parameters, defined as an increase in 25(OH)D₃ concentration in the blood serum. The authors recommend the supplementation of vitamin D₃ depending on the general condition and the level of deficiency as outlined in Table 1.

A limitation of our study may be the fact that it was conducted in the summer period, when the synthesis of vitamin D in the body is higher. This may have had an impact on obtaining a trend rather than a statistically significant result regarding the level of vitamin D and the frequency of periodontal disease. Another element that could have affected the results was the collection of blood from the ulnar vein and not from the capillaries.

Table 1. Recommended vitamin D supplementation depending on the level of deficiency


Vitamin D level [ng/mL]	Supplementation
>30 (normal level)	4,000 IU per day throughout the year
20–30	8,000 IU per day, follow-up after 3 months
<20	10,000 IU per day, follow-up after 3 months

Conclusions

The analysis of the current results has shown that decreased levels of vitamin D are present in a large proportion of dental patients, and that an increase in the incidence of periodontal disease is likely associated with a decrease in the level of vitamin D. The level of vitamin D tends to decrease with age and in persons with periodontal disease. Thus, vitamin D concentration should be determined before periodontal procedures and, if needed, supplementation should be administered.

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Effect of metal ions released from orthodontic mini-implants on osteoclastogenesis

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Conflict of interest

None declared

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Abstract

Background. Orthodontic mini-implants can undergo corrosion and the release of metal ions can affect cellular behavior. Osteoclasts are involved in orthodontic tooth movement and implant stability. Osteoclasts and their precursors can be exposed to metal ions released from orthodontic mini-implants.

Objectives. This study aimed to investigate the effect of metal ions released from orthodontic mini-implants on human osteoclastogenesis.

Material and methods. Stainless steel and titanium alloy mini-implants were separately immersed in culture media for 14 days (days 1–14), and then moved to new media for a further 14 days (days 15–28). The concentration of the released metal ions was measured. Osteoclast precursors derived from human CD14⁺ monocytes were cultured in these media and in a control medium without mini-implant immersion. Cell viability, the number of osteoclasts and the area of resorption were investigated.

Results. A higher concentration of metal ions was detected during the first 14 days as compared to the control. The concentration of these metal ions then declined after this period. The viability of osteoclast precursors was not affected by the released metal ions. There was a significant reduction in the number of osteoclasts when cultured in the medium with the titanium alloy mini-implants immersed for days 1–14. The area of resorption was also significantly reduced in this group. The media with the titanium alloy mini-implants immersed for days 15–28 and with the stainless steel mini-implants immersed for both study periods did not show statistically significant changes in the number of osteoclasts.

Conclusions. Metal ions were released from orthodontic mini-implants in the early period and declined thereafter. Metal ions released from titanium mini-implants in the early period inhibited osteoclastogenesis, while metal ions from stainless steel mini-implants had no effect on osteoclast differentiation.

Keywords: cytotoxicity, biocompatibility, osteoclast, metal ion, orthodontic mini-implant

Introduction

Orthodontic mini-implants, also known as orthodontic miniscrews or temporary anchorage devices, have increased in popularity for use in orthodontic treatment in recent decades. Mini-implants are placed into bone during orthodontic treatment to act as skeletal anchorages in order to provide tooth movement, which is impossible with the use of tooth-borne anchorages.¹

Materials commonly used for orthodontic mini-implants are titanium or titanium alloy and stainless steel.² All metals that are in contact with a biological fluid can undergo corrosion and metal ions are released into the biological system.^{3–5} Orthodontic mini-implants undergo corrosion. Metal ions released from orthodontic mini-implants can be detected locally in the saliva of patients after mini-implant placement, and also systemically in remote organs in animal studies.^{6,7}

Metal ions can affect various cell types in the human body, including osteoclasts.^{8,9} When mini-implants are placed into bone, osteoclasts and their precursors can be exposed to the released metal ions. Osteoclasts are bone-resorbing cells involved in implant stability; thus, any disturbance of osteoclast activity can be related to implant failure.^{10,11} Osteoclasts are crucial in orthodontic tooth movement and the inhibition of osteoclasts may compromise tooth movement. In certain situations, the tooth is expected to move toward the mini-implant during the treatment period, such as in retromolar placement for distalization.¹² Prolonged effects of metal ions released from mini-implants on osteoclastogenesis may affect tooth movement in such cases. In a previous report, mini-implants were used to create micropores in bone to accelerate tooth movement.¹³ In such circumstances, the teeth are quickly moved toward the sites of the previously placed mini-implants. It is important to recognize and better understand factors related to mini-implant stability as well as to determine ways of utilizing mini-implants for different purposes. Therefore, this study aimed to investigate the effect of metal ions released from orthodontic mini-implants on osteoclastogenesis during different periods of time.

Material and methods

Preparing culture media with mini-implant immersion

Titanium alloy (Ti₆Al₄V) mini-implants (Renew Biocare Corporation Asia Pacific, Taipei, Taiwan) and stainless steel mini-implants (Bio-Ray Biotech Instrument Co., Ltd., Taipei, Taiwan) were used in this study. The titanium alloy consisted of titanium-based metal with 6% aluminum, 4% vanadium and trace amounts of other elements.

The stainless steel mini-implants contained 63% iron, 17% chromium, 14% nickel, 3% molybdenum, 2% manganese, and 1% other elements.

The titanium alloy and stainless steel mini-implants were separately immersed in culture media containing α -MEM (Minimum Essential Medium) (HyClone, Logan, USA) at a ratio of 1 mL/0.1 g and 1 mL/0.2 g, respectively. These ratios are recommended by the International Standards Organization (ISO) and the Japanese Ministry of Health, Labour and Welfare. Both kinds of mini-implants were immersed in the media for 14 days (days 1–14), and then moved to new media for another 14 days (days 15–28). The media were kept in a humidified atmosphere at 37°C during the immersion periods. The media from each period were then collected and divided into 2 parts. One was used to measure the concentration of metal ions, and the other was used to culture osteoclasts in order to determine their number and cell viability, and the area of resorption.

Measurement of metal ion release

The concentration of metal ions was detected using inductively coupled plasma mass spectrometry (ICP-MS) (iCAP™ RQ ICP-MS; Thermo Fisher Scientific, Bremen, Germany). The instrument parameters are shown in Table 1. The concentrations of iron, chromium and nickel ions – the 3 main components of stainless steel mini-implants – were measured in the media in which the stainless steel mini-implants were immersed. The concentrations of titanium, aluminum and vanadium ions – the components of titanium alloy mini-implants – were measured in the media in which the titanium alloy mini-implants were immersed.

Osteoclast differentiation

Human osteoclast precursors were derived from monocytes obtained from the peripheral blood of healthy donors. The protocol was approved by the Ethics Committee of the Research Institute of Rangsit University, Thailand (RSEC 33/2559) and informed consent was obtained from the subjects. Briefly, 50 mL of peripheral venous blood was collected from each healthy donor. Peripheral blood mononuclear cells (PBMCs) were separated through density gradient centrifugation, using Histopaque®-1077 (Sigma-Aldrich, St. Louis, USA). CD14⁺ monocytes were further sorted

Table 1. General operational conditions of inductively coupled plasma mass spectrometry (ICP-MS)

Parameter	Value
Plasma power	1,550 W
Cool flow	14 L/min
Auxiliary flow	0.8 L/min
Nebulizer flow	1.043 L/min
Dwell time	0.1 s

using human CD14 MicroBeads (Miltenyi Biotec, Auburn, USA). The cells were then plated on a 96-well plate at a density of 10^5 cells per well to determine cell viability and the number of osteoclasts. For the detection of a resorption pit, 7×10^4 cells were plated in each well of a 96-well plate containing dentin slices, 5 mm in diameter, which were prepared from cylindrical elephant ivory cut to a thickness of 300 μm , as previously described.¹⁴ The cells in each well were maintained in 50 μL of the culture media in which the mini-implants were previously immersed or in a medium without mini-implant immersion, which served as the control. All of the culture media were supplemented with 10% fetal bovine serum (FBS) (Gibco™, Thermo Fisher Scientific, Rochester, USA), 0.2 mM GlutaMAX™ (Gibco), 100 U/mL penicillin (Gibco), 100 mg/mL streptomycin (Gibco), 0.25 $\mu\text{g}/\text{mL}$ amphotericin B (Gibco), 25 ng/mL recombinant human receptor activator of nuclear factor kappa-B ligand (rh-RANKL) (PeproTech Inc., Rocky Hill, USA), and 25 ng/mL recombinant human macrophage-colony stimulating factor (rhM-CSF) (PeproTech Inc.). The media were changed every 2–4 days. At least 3 different lines of osteoclast precursors were used and at least 2 independent experiments were performed.

Cell viability assay

The viability of osteoclast precursors was determined after 20 h of cell culture. Briefly, the cells were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (USB Corporation, Cleveland, USA) for 40 min at 37°C with 5% CO₂. Formazan crystals were dissolved with a solubilization agent containing 1:9 dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany) and glycine buffer (0.1 M glycine/0.1 M sodium chloride; pH 10). The absorbance of the solution at 570 nm was then measured using a microplate reader (ELx800™; BioTek Instruments, Winooski, USA).

Determination of the number of osteoclasts

After 14 days of differentiation, the cells were stained for tartrate-resistant acid phosphatase (TRAP) and the number of osteoclasts in each sample was determined. Briefly, the cells were fixed with 10% neutral buffered formalin followed by washing with 95% ethanol. The cells were then stained for TRAP with 0.1 mg/mL naphthol AS-MX phosphate (Sigma-Aldrich) and 0.5 mg/mL fast red violet LB salt (Sigma-Aldrich) in 50 mM sodium acetate buffer (pH 5.0) containing 50 mM sodium tartrate for 20 min. After washing, the cells were visualized under bright-field microscopy. Images were obtained from at least 3 fields for each culture well with the use of $\times 100$ stage objectives (Olympus DP72; Olympus, Tokyo, Japan). Cells that were TRAP-positive and contained at least 3 nuclei were counted as osteoclasts.

Determination of resorptive activity

After 14 days of osteoclast differentiation, the cells were removed from the dentin slices by sonication in 25–30% ammonium hydroxide. After washing, the dentin slices were stained with 1 mg/mL toluidine blue and visualized under bright-field microscopy. Images were obtained from at least 3 fields for each dentin slice under $\times 100$ stage objectives (Olympus DP72; Olympus). The area of resorption was measured using the measurement tool in the cellSens software (Olympus).

Statistical analysis

The mean concentration of metal ions in the media in which the mini-implants were immersed was compared to the control using the one-sample *t* test. Cell viability was analyzed by comparing the mean absorbance value among all groups using the one-way analysis of variance (ANOVA). The number of osteoclasts was calculated as fold changes compared to the control from the same precursor lines. The mean fold changes in the number of osteoclasts were compared among all groups using the one-way ANOVA. The least significant difference (LSD) post-hoc test was performed for any significant difference identified with ANOVA. The resorption area was calculated as a fold change compared to the control from the same precursor lines. The mean fold change in the resorption area in the test group was compared to that of the control using Student's *t* test.

Results

Metal ions released from orthodontic mini-implants

The presence of metal ions in the media in which the mini-implants of either material were immersed was confirmed. The concentrations of iron, chromium and nickel ions were significantly higher in the medium with the stainless steel mini-implants immersed for days 1–14 as compared to the control ($p < 0.01$, $p < 0.05$ and $p < 0.01$, respectively) (Table 2). The medium with the titanium alloy mini-implants immersed for days 1–14 had significantly higher concentrations of titanium and vanadium ions as compared to the control ($p < 0.01$) (Table 2). This indicates a substantial release of metal ions from the mini-implants of either material during this period. At days 15–28, the level of metal ions declined until no significant difference was observed for all metal ions except titanium ions; their concentration was significantly higher than in the control medium ($p < 0.01$). We were unable to detect any increase in the concentration of aluminum ions after mini-implant immersion in either period due to a high background amount of aluminum ions in the media. Taken together,

Table 2. Concentration of metal ions in the control medium without mini-implant immersion and the media in which the stainless steel and titanium alloy mini-implants were immersed [$\mu\text{g/L}$, ppb]

Group	Fe	Cr	Ni	Group	Ti	V	Al
control	<0.1	<0.02	3.50	control	1.50	<0.02	138.50
SS 1–14	9.67**	5.67*	8.83**	Ti 1–14	23.17**	3.83**	134.33
SS 15–28	<0.1	<0.02	2.67	Ti 15–28	15.33**	0.33	117.50

SS – stainless steel; Ti – titanium alloy; 1–14 – days 1–14 of immersion; 15–28 – days 15–28 of immersion; * $p < 0.05$ compared to the control; ** $p < 0.01$ compared to the control.

the results confirm that metal ions were released from the orthodontic mini-implants of either material. The release of metal ions was higher during the initial period and decreased over time, with a prolonged release of titanium ions from the titanium alloy mini-implants.

Viability of osteoclast precursors

The number of viable osteoclast precursors indicated by the absorbance measured after the MTT assay was not statistically different while comparing the control medium, the media in which the titanium alloy mini-implants were immersed and the media in which the stainless steel mini-implants were immersed ($p \geq 0.05$) (Fig. 1). This indicates that the presence of metal ions from both the stainless steel and titanium alloy mini-implants used in this study was not cytotoxic to osteoclast precursors. Thus, metal ions influenced the ability of osteoclasts to differentiate rather than caused cell death, which is confirmed by the results presented below, regarding the number and resorption ability of osteoclasts.

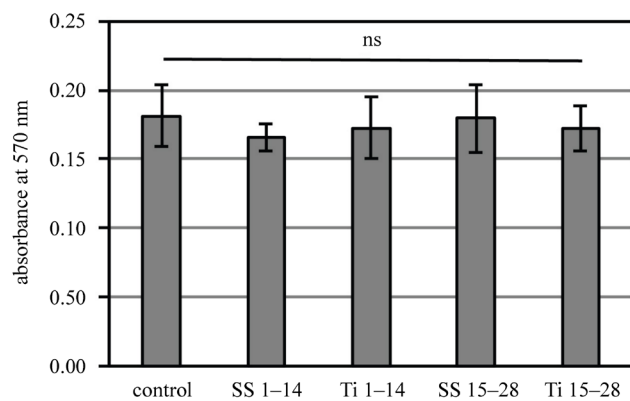


Fig. 1. Viability of osteoclast precursors. The absorbance measured after the MTT assay indicated no statistically significant differences in the number of viable cells between all media groups

ns – not significant ($p \geq 0.05$).

Data presented as mean \pm standard deviation ($M \pm SD$) ($n = 3$).

Effect on osteoclastogenesis

There was a significant reduction in the number of osteoclasts when cultured in the medium with the titanium alloy mini-implants immersed for days 1–14 ($p < 0.05$).

For the medium with the stainless steel mini-implants immersed for days 1–14, although there was a tendency toward a reduction in the numbers of osteoclasts, no significant change was observed ($p \geq 0.05$) (Fig. 2).

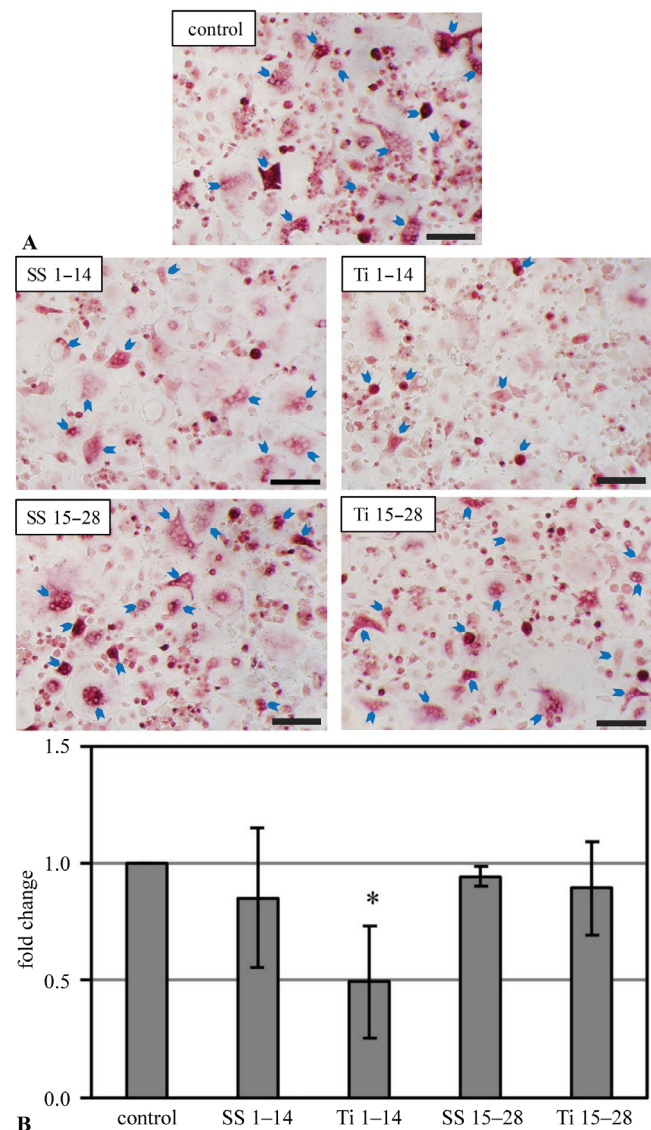


Fig. 2. Changes in the number of osteoclasts. A. Representative images of the TRAP staining of osteoclasts in the control and mini-implant immersion media after 14 and 28 days of differentiation. B. The number of osteoclasts in the mini-implant immersion media was calculated as fold changes compared to the control. Fold changes in the osteoclast number compared to the control in SS 1–14, Ti 1–14, SS 15–28, and Ti 15–28 were 0.85 ± 0.30 , 0.49 ± 0.24 , 0.94 ± 0.05 , and 0.89 ± 0.20 , respectively

* statistically significant ($p < 0.05$).

Data presented as $M \pm SD$ ($n = 4$).

For days 15–28, no significant change in the osteoclast number was noted for the mini-implants of either material ($p \geq 0.05$) (Fig. 2).

The ability of osteoclasts to resorb the mineralized tissue was further researched in the medium with the titanium alloy mini-implants immersed for days 1–14; in this group there was a significant decrease in the osteoclast number. It was found that the area of resorption was also significantly reduced in this case ($p < 0.05$) (Fig. 3). Collectively, the results indicate that there was a significant suppression of osteoclastogenesis in the presence of metal ions released from the titanium alloy mini-implants at days 1–14.

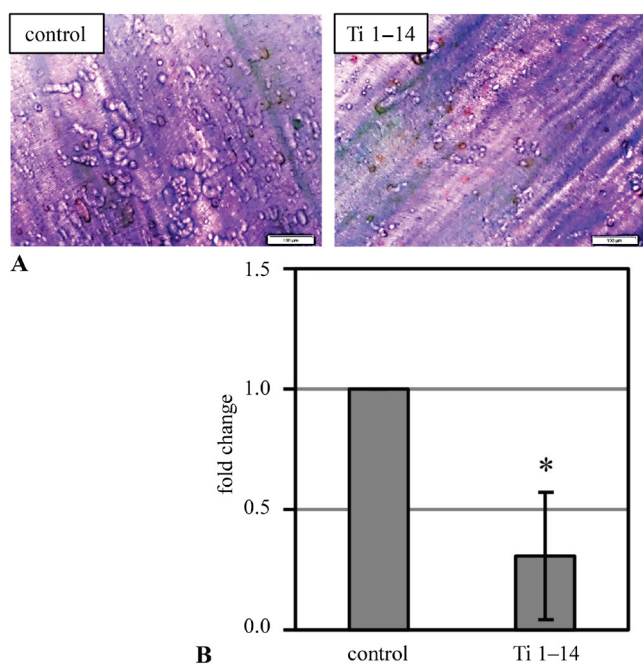


Fig. 3. Changes in the area of resorption. A. Representative images of resorption pits on the dentin slices in the control medium compared to the medium with the titanium alloy mini-implants immersed for days 1–14. B. The area of resorption of the osteoclasts cultured in the medium with the titanium alloy mini-implants immersed for days 1–14 was calculated as a fold change compared to the control. A fold change in the area of resorption in the Ti 1–14 group compared to the control was 0.31 ± 0.26 .

* statistically significant ($p < 0.05$).
Data presented as $M \pm SD$ ($n = 3$).

Discussion

The results of this study confirm that metal ions were released from both stainless steel and titanium alloy orthodontic mini-implants. After the initial release, it is possible that soluble metal ions transformed into solid corrosion precipitates, which may have lowered the concentrations of metal ions detected in the solution over time. However, a previous study indicated that within a 28-day period, the level of metal ions released from titanium alloy mini-implants continued to increase with longer immersion durations from 1 day to 28 days.¹⁵

In this study, the release of metal ions from orthodontic mini-implants was found to be higher when the mini-implants were initially in contact with the media and decreased over time. This could be due to the formation of a thin barrier film on the surface of the initially corroded mini-implants, which served to prevent further corrosion of deeper metal atoms.¹⁶ Nonetheless, since mechanical force may disrupt this barrier layer,¹⁶ it should be noted that when force is applied to mini-implants in the patient's mouth, the release of metal ions may continue beyond the periods investigated in this study.

The stainless steel mini-implants used in this study were made of type 316L stainless steel from the American Iron and Steel Institute. This material is recommended by the American Society for Testing and Materials (ASTM International) for use as implant material. Stainless steel 316L contains molybdenum and has a low carbon content, which minimizes intergranular corrosion, and also makes the material more resistant to chlorine-bearing solutions.²

The presence of metal ions at high concentrations can be cytotoxic to cells,^{17–19} but with biocompatible material, the release of metal ions should not affect cell viability. This study demonstrated that the concentrations of metal ions released from the orthodontic mini-implants of either material were within a non-cytotoxic range for osteoclast precursor cells. This result is consistent with previous studies which investigated the biocompatibility and cytotoxicity of orthodontic miniscrews in L929, HaCaT, HGF, and U2OS cells.^{17,20} They reported that there was no cytotoxicity due to titanium mini-implants when in contact with a physiological pH solution.^{17,20}

Different metal ions may have different effects on osteoclasts. However, this study was not designed to separately evaluate the effect of each type of metal ions. Instead, we employed the direct elution of orthodontic mini-implants, so the effect on osteoclasts was from the combination of various metal ions released in a proportion that corresponded to the corrosion rate of each ion type. This should represent the release of metal ions in clinical situations, in which mini-implants are inserted into the patient's bone.

The results of the present study indicate that metal ions released from the titanium alloy mini-implants during the initial period (days 1–14) had a suppressive effect on osteoclastogenesis. These results support the findings of previous studies, which found that titanium ions might reduce the number of osteoclasts and resorption pits.^{21,22} Vanadium ions were shown to have an inhibitory effect on osteoclasts in previous studies.^{18,22} Metal ions released from the titanium alloy mini-implants during the later period (days 15–28) did not affect the number of osteoclasts. This could be due to the fact that the concentrations of metal ions found in this period were too low to have an effect on osteoclasts.

The effect of iron and chromium ions – the components of stainless steel – on osteoclasts has been reported previously with conflicting results. Iron ions were

reported to stimulate osteoclast formation by Jia et al.,²³ yet significantly reduced the osteoclast number in a study by Rousselle et al.²² Chromium was found to have no effect on osteoclasts when using animal cells,²¹ whereas a study using human PBMCs found an inhibitory effect of Cr⁶⁺ and a biphasic effect of Cr³⁺ on osteoclasts.²⁴ In the present study, metal ions released from the stainless steel mini-implants in both study periods were found not to affect the number of osteoclasts. This could result from each of these metal ions having no effect on osteoclasts or the effect on osteoclasts was neutralized, since some of these metal ions may have an inhibitory effect, while others have a stimulatory effect. Additionally, since the concentrations of the ions of each metal producing the effect on particular cells is varied, it is also possible that the level of metal ions released from the stainless steel mini-implants did not reach a sufficient concentration to affect osteoclasts.

The suppression of osteoclastogenesis by metal ions from the titanium alloy mini-implants did not result from the death of monocyte precursors according to cell viability results (Fig. 1). Therefore, a reduction in the osteoclast number must have been a result of disturbance in the differentiation and maturation process of osteoclasts, which is regulated by various mechanisms. Zinc has been reported to suppress osteoclasts by inhibiting the Ca²⁺-calcineurin-NFATc1 signaling pathway.²⁵ Meanwhile, magnesium has been shown to inhibit osteoclasts through the inhibition of the nuclear factor kappa B (NF-κB) pathway.²⁶ However, the mechanisms underlying the inhibition of osteoclasts by metal ions from titanium mini-implants require further investigation.

Changes in osteoclastic activity may affect mini-implant stability. It has been reported that the local delivery of bisphosphonate – a drug that inhibits osteoclastic activity – can enhance mini-implant stability.²⁷ Therefore, the results of the present study suggest that metal ions released from the titanium alloy mini-implants during the early period may be one factor that promotes the stability of orthodontic mini-implants according to their inhibiting effect on osteoclastogenesis.

Early mini-implant loading is beneficial in terms of reducing treatment time; however, stability is of concern in instances of early loading. Previous studies reported that the early loading of orthodontic force onto mini-implants did not compromise their stability as compared to delayed loading.^{28,29} The results of this study also support the early loading of the titanium alloy mini-implants, since a decrease in osteoclastic activity should minimize the likelihood of the loosening of mini-implants during the first 2 weeks.

Recently, orthodontic mini-implants have been reported to be used for micro-osteoperforation to accelerate tooth movement.^{13,30} In this procedure, mini-implants are placed and removed at the alveolar bone adjacent to the teeth to create microperforations prior to rapid tooth

movement toward the mini-implant sites. Since the stimulation of osteoclasts is expected at mini-implant placement sites following this procedure, stainless steel mini-implants would be preferred according to the results of this study.

After 2 weeks, the release of metal ions from orthodontic mini-implants decreased until no effect on osteoclasts was observed for the mini-implants made of either material. According to this result, in certain cases in which the teeth must be moved toward mini-implants during treatment, such as in retromolar placement for molar distalization, after a 2-week period, metal ions from the mini-implant should no longer affect osteoclasts and tooth movement.

Conclusions

This study demonstrated that metal ions were released from stainless steel and titanium alloy orthodontic mini-implants at non-cytotoxic levels for osteoclast precursor cells. The release of these metal ions was found to be higher during the initial period, but then declined over time. Finally, metal ions released from titanium mini-implants during the initial period inhibited osteoclastogenesis, while metal ions from stainless steel mini-implants were found not to affect osteoclast differentiation.

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Protective effects of caffeic acid phenethyl ester on the heart in experimental periodontitis against oxidative stress in rats

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Conflict of interest

None declared

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Abstract

Background. Caffeic acid phenethyl ester (CAPE) may be considered as alternative treatment for periodontitis and benefit the heart by way of its ameliorative effects.

Objectives. The aim of the study was to evaluate the effects of CAPE on cytokine levels and the oxidative status in the serum and heart tissue in a rat model of periodontitis.

Material and methods. Experimental animals were randomly assigned to 3 groups: control group (C; $n = 8$); periodontitis group (P; $n = 10$); and periodontitis + CAPE group (PC; $n = 10$). Caffeic acid phenethyl ester, at a dose of 10 $\mu\text{mol/kg/day}$, was administered by intraperitoneal injection over a 14-day period. Interleukin (IL)-1 β , IL-10 and tumor necrosis factor- α (TNF- α) were assessed in the serum. Glutathione (GSH), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were assessed in both the serum and the heart tissue homogenate.

Results. Increased IL-1 β , IL-10 and TNF- α serum levels were observed in the P group ($p < 0.05$). Caffeic acid phenethyl ester significantly decreased alveolar bone loss (ABL) and cytokine levels in the PC group ($p < 0.05$). Malondialdehyde, one of the strongest oxidants, was significantly decreased in the PC group as compared to the P group ($p < 0.05$). In both the serum and the heart tissue homogenate there were no differences in MDA levels between the PC and C groups. Furthermore, CAPE significantly increased GSH and GSH-Px levels in the serum and heart tissue ($p < 0.05$).

Conclusions. Caffeic acid phenethyl ester has beneficial effects on the tissues affected by periodontitis.

Keywords: free radicals, heart, caffeic acid phenethyl ester, inflammatory process, periodontal damage

Cite as

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Introduction

Caffeic acid phenethyl ester (CAPE) is an active polyphenolic compound and a valuable element of propolis, which is a component of honey. It has numerous health benefits due to its antioxidative, antiproliferative, antitumoral, anti-inflammatory, immunomodulatory, and anti-neoplastic properties. An epidemiological study reported a reduction of blood pressure and the lessening of cardiovascular problems, mainly related to coronary heart disease, i.e., antithrombotic, anti-ischemic and vasorelaxant effects, following a regular intake of honey.¹ The timely cardioprotective activity of CAPE, which decreased the diversion of xanthine oxidase (XO) and adenosine deaminase (ADA) as well as had a direct antioxidant effect, has been demonstrated in short-term myocardial ischemia in rats.² Many studies have discussed the links between CAPE and mitogen-activated protein kinase (MAPK) pathways, which are an important part of treating cardiac disease.^{3,4} Caffeic acid phenethyl ester inhibits the stimulation of p38 MAPK, the activity of caspase-3, and the production of proinflammatory cytokines (interleukin (IL)-1 β and tumor necrosis factor-alpha (TNF- α)). It also reduces cardiomyocyte apoptosis in cardiac tissues.⁵ The anti-inflammatory effect of CAPE is related to decreases in c-Jun N-terminal kinase, nuclear factor-kappa B (NF- κ B) and cyclooxygenase (COX)-2 expression.⁶ Thus, the CAPE-based protection of cardiac myocytes is likely accomplished through inhibition – not only through the inhibition of inflammatory signaling, but also via the inhibition of cell death.⁶ Another useful effect of CAPE on cardiomyocyte cells is the control of accumulation of reactive oxygen species (ROS). Reactive oxygen species play a pivotal role in tissue harm and diseases. Several pathological conditions, such as cardiovascular diseases (CVD) (atherosclerosis, hypertension and congestive failure), can be stimulated when intracellular redox homeostasis is impaired.^{7,8} Cells always need oxygen for energy, which is obtained through oxidative phosphorylation. The production of adenosine triphosphate (ATP) begins with the reaction of 4 electrons and 4 protons joining O₂ to form 2 water molecules (H₂O). This process is sensitive, because, if a molecule of O₂ gets only 1 electron, it is modified into a superoxide anion (O₂⁻). Hydrogen peroxide (H₂O₂), hydroxyl radical (OH[•]) and peroxynitrite (ONOO⁻) are examples of highly reactive ROS that can afford to keep more electrons as well as 4 protons to form H₂O. Antioxidant defense mechanisms act to prevent the pathophysiological damage caused by ROS. Nevertheless, oxidative stress can induce many different diseases, such as CVD (e.g., atherosclerosis or hypertension), diabetes mellitus, neurodegenerative disorders, rheumatoid arthritis, and periodontitis, as well as ageing.^{9,10}

Periodontitis is a chronic inflammatory disease characterized by the destruction of periodontal tissues, such as connective tissue, bone, and teeth.¹¹ Host and bacterial challenges are the key factors in the progression of peri-

odontal disease and responses to systemic inflammation.¹² In fact, physiological ROS, stimulated by immune cells, have antibacterial effects that support the immune regulation and host defense. Cytotoxic effects on cell growth and the cell cycle, the oxidative destruction of DNA and proteins, and the induction of gingival fibroblast apoptosis can all be stimulated by excessive ROS. When periodontal tissue destruction begins, the main role of ROS is as intracellular signaling molecules during osteoclastogenesis. As a result, bone destruction is indirectly induced.^{10,13}

The triad of inflammation, oxidative stress and periodontitis has often been emphasized in previous studies. Inflammation and oxidative stress are the main elements of this pattern, and can also have pathological effects on other tissues. For example, periodontitis can have a negative effect on heart tissue because of increased pro-inflammatory cytokine levels and systemic oxidative stress. To some degree, oxidative stress can be blocked by autoimmune protective mechanisms, starting with the production of cytoprotective enzymes.¹⁰ One of these protective mechanisms may involve CAPE, as assessed in the present study. Specifically, we evaluated the antioxidant and anti-inflammatory effects of CAPE on cytokine levels and the oxidative status in the serum and heart tissue.

Material and methods

Animals

Twenty-eight male Wistar albino rats weighing approx. 200 \pm 20 g were individually housed in plastic cages. The rats were kept in standard environmental conditions, fed with standard rat food and had tap water ad libitum. The room temperature was set at 24.0 \pm 0.6°C and the cages were kept in a 12h/12h dark/light cycle (the lights were turned on at 07:30 a.m.). The animals were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by Süleyman Demirel University, Isparta, Turkey (study approval No. 26-09/12).

Induction of periodontitis

The experimental animals were randomized into 3 groups: control group (C; $n = 8$); periodontitis group (P; $n = 10$); and periodontitis + CAPE group (PC; $n = 10$). The ligature-induced periodontitis model is a consistent and safe method in which the accumulated plaque evokes periodontitis and stimulates the host response in the den-togingival region.^{14,15} Periodontitis is induced by placing ligatures on molar teeth. The waiting period for the periodontal reaction to begin should be less than 15 days.¹⁴ In this study, the histomorphometric analysis revealed that periodontal destruction successfully occurred in the experimental groups after 14 days (Fig. 1).

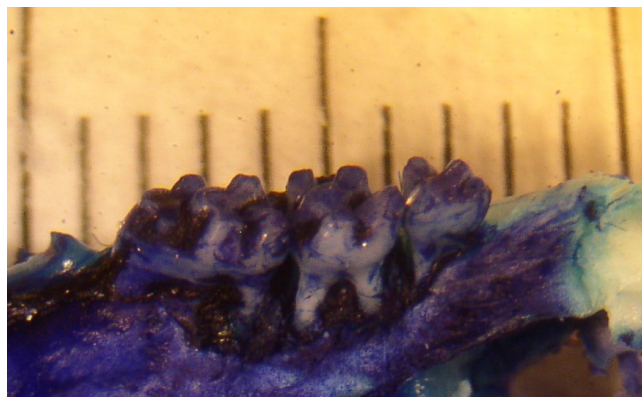


Fig. 1. Microscopic image of bone loss magnification $\times 4$.

Experimental periodontitis was induced under general anesthesia, using intramuscular ketamine (90 mg/kg) and xylazine (10 mg/kg) injection (Pfizer, Tadworth, UK).¹¹ Sterile 3/0 silk ligatures (Doğsan, Istanbul, Turkey) were subgingivally wrapped around maxillary second molars in both groups for a duration of 14 days.¹⁴ Caffeic acid phenethyl ester (Sigma-Aldrich, St. Louis, USA)¹² was administered at a dose of 10 $\mu\text{mol/kg/day}$ via intraperitoneal injection for 14 days.¹⁶ All chemicals were of analytical grade.

Histomorphometric analysis

After the rats were sacrificed under general anesthesia, the maxillae and hearts were resected and immediately fixed in H_2O_2 for 24 h. The right maxillary halves were used for histomorphometric evaluation and stained with 1% methylene blue (Ceristain®; Merck, Darmstadt, Germany) (1 g/100 mL) diluted with water for 60 s to fix the cemento-enamel junction (CEJ). The distance between CEJ and the alveolar bone crest (ABC) was measured at 3 buccal points under a stereomicroscope (DP-Soft 3.2, Olympus Europa, Hamburg, Germany) at $\times 40$ magnification. Morphometric alveolar bone loss (ABL) was recorded using standardized digital photography (Leica MZ6; Leica, Wetzlar, Germany) and the images were analyzed using the ImageJ software, v. 1.46r (National Institutes of Health, Bethesda, USA).

Heart tissue homogenization

The hearts were cleaned with cold isotonic water, packaged in aluminum foil, and then stored at -85°C in a deep freezer until analysis. Homogenization was performed at 16,000 rpm. The samples were placed in a refrigerated centrifuge (Kubota, Tokyo, Japan) and centrifuged at 3,220 rpm for 30 min at 6°C . The supernatant 1/1 (v/v) mixture of chloroform/ethanol (3/5, v/v)¹⁷ was transferred to glass tubes, vortexed, and then centrifuged at 3,220 rpm for 40 min at 4°C .

Determination of serum cytokine levels

Centrifugation was used to separate the serum samples (at 1,500 rpm for 10 min at 4°C). The samples were then poured into Eppendorf tubes and stored at -20°C until testing began. Interleukin-1 β , IL-10 and TNF- α levels were assessed using enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, Vienna, Austria) with a microplate reader (Infinite® M200; Tecan, Männedorf, Switzerland). The concentrations of IL-1 β , IL-10 and TNF- α were assayed from a standard curve and following the manufacturer's recommendations. The minimum assay detection limit was 4 pg/mL for IL-1 β , 1.5 pg/mL for IL-10 and 11 pg/mL for TNF- α .

Determination of MDA, GSH and GSH-Px levels

The method of Placer et al. was used for the analysis of lipid peroxidation (LPO) levels.¹⁸ Briefly, a pink color is a marker produced by mixing thiobarbituric acid (TBA) with MDA to design a colored MDA-TBA adduct. Lipid peroxidation levels, as presented by MDA, were observed using a spectrophotometric assay (Shimadzu UV-1800, Shimadzu Corp., Kyoto, Japan) at a wavelength of 532 nm.

The samples were assessed for GSH at 412 nm, using the method of Sedlak and Lindsay.¹⁹ Glutathione peroxidase spectrophotometric assessments were performed at 412 nm at 37°C , based on the Lawrence and Burk technique.²⁰ Malondialdehyde, GSH and GSH-Px levels were evaluated in the serum and heart tissue. The whole experimental protocol is presented in Fig. 2.

Statistical analysis

All statistical analyses were performed using the SPSS Statistics for Windows software, v. 17.0 (SPSS Inc., Chicago, USA). Group significance levels were assessed with the one-way analysis of variance (ANOVA). The results were reported as means (*M*) and standard deviations (*SD*). The Shapiro-Wilk test was used to assess the normal distribution of the variables. A *p*-value < 0.05 indicated statistical significance. The independent *t* test was used to determine differences between the groups.

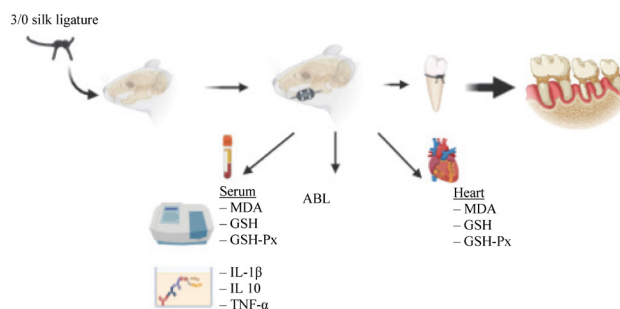


Fig. 2. Experimental protocol

Results

Both experimental groups showed periodontitis symptoms. Alveolar bone loss occurred more commonly in the P group than in the PC group ($p < 0.05$). Inflammatory infiltration was observed in the P group as increased IL-1 β , IL-10 and TNF- α serum levels as compared to the control (all $p < 0.05$). The 3 cytokines were downregulated due to the administration of CAPE. Hence, cytokine levels were downregulated in the PC group as compared to the P group ($p < 0.05$). Additionally, in the PC group, the antioxidative activity of CAPE was observed through the highest GSX and GSH-Px serum levels. Since periodontitis was induced without the administration of any agent, these 2 antioxidants were lower in the P group as compared to other groups ($p > 0.05$). The serum levels of the strongest oxidative marker – MDA – were significantly increased in the P group. For the other 2 groups, they were higher in the PC group than in the C group, although the difference was not statistically significant ($p > 0.05$).

Indeed, a significant relationship was observed between periodontitis, ROS and the affected heart in this study. The heart analysis almost repeated the pattern of the serum oxidant and antioxidant results. The highest MDA heart levels were observed in the P group and the lowest in the C group. However, the differences were not statistically significant ($p > 0.05$). These values returned to the original levels after the administration of CAPE. Additionally, GSH and GSH-Px heart levels were lower in the P group than in the C and PC groups ($p < 0.05$). Of course, the PC group had the highest levels due to oxidative stress. After the administration of CAPE, both antioxidant parameters were improved in the PC group ($p < 0.05$). All group comparisons are shown in detail in Table 1.

Discussion

The objective of this study was to reveal the effect of CAPE on cytokine levels and the oxidative status in the serum and heart tissue, using a rat periodontitis model. Our results demonstrated that CAPE is a good antioxidant and anti-inflammatory agent for decreasing the progression of periodontitis and its related effects on the heart in rats.

Caffeic acid phenethyl ester produces the most significant anti-inflammatory and antioxidant effects of all extracts of propolis.²¹ In recent studies using CAPE, it has been reported that CAPE has a protective effect with regard to cellular mechanisms in which oxidative stress is increased due to inflammation, and thus CAPE can protect many cell groups, including nerve cells, against oxidative damage.^{22,23} Caffeic acid phenethyl ester inhibits the lipoxygenase pathway of arachidonic acid metabolism and ROS production during inflammation.²⁴ It is a potent

inhibitor of NF- κ B, which plays an important role in bone destruction. It has been found that these properties are helpful in healing bone defects and preventing alveolar bone loss.^{25,26}

Hence, CAPE may be a beneficial additive agent in treating many diseases. Various studies have shown that CAPE and propolis reduce pro-inflammatory cytokine levels.^{27,28} Caffeic acid phenethyl ester was shown to relieve inflammatory pressure by decreasing the expression of NF- κ B and COX-2 in an obese mouse model.²⁹ The cardioprotective effects of CAPE following cardiac injury have been found to consist in decreasing apoptosis, oxidative stress and cardiomyocyte damage after suppressing pro-inflammatory cytokines, especially TNF- α expression.³⁰ In addition, the oxidative stress induced by inflammation can provoke various pathological processes in the heart. The administration of CAPE was found to decrease MDA levels in aged rat heart.³¹ Increases in oxidative stress and the inflammatory response can result in CVD or negatively affect their course. Different studies have reported a possible cross-connection between MDA and heart disease via effects on genetics and cellular membranes in heart tissue.³² Tomofuji et al. reported that increased LPO products in periodontal inflammation contributed to oxidative DNA damage in the heart.³³

Several studies have indicated that CAPE might be a regeneration alternative for bone resorption, as it improves the bone healing process and prevents receptor activator for NF- κ B ligand (RANKL)-induced osteoclastogenesis. Previous studies have reported stimulating effects of antioxidants on bone healing in periodontitis.²⁸ The formation of OH \cdot , H $_2$ O $_2$ and O $_2^{\cdot-}$ radicals in periodontal tissue is mainly caused by neutrophils and macrophages. The O $_2^{\cdot-}$ radical is thought to be associated with osteoclastic activity and bone resorption. It starts a dangerous attack against the host response and causes DNA damage due to enzymatic oxidation, vasodilation, the release of proinflammatory cytokines, such as IL-1, IL-6, IL-8, TNF- α , TNF- β , and interferon, through NF- κ B-based signal transduction pathways, and LPO activation related to bone resorption. Uncontrolled LPO can result in oxidative stress that severely damages cell integrity.¹⁷

Many studies have highlighted how an increased amount of pro-inflammatory cytokines can cause periodontal destruction.^{11,25} The administration of CAPE has been shown to slow down alveolar bone resorption and to decrease pro- and anti-inflammatory cytokine serum levels in periodontitis.²⁸ Caffeic acid phenethyl ester was also shown to increase the filling rates of calvaria bone defects in rats.²⁵ Our findings are similar to those of previous research. The highest amount of ABL occurred in the P group and bone gain started after the administration of CAPE. Malondialdehyde levels increased and the activity of basic antioxidant enzymes, such as GSH and GSH-Px, decreased in experimental periodontitis.¹⁵

Table 1. Comparison of alveolar bone loss (ABL), and cytokine, oxidant and antioxidant levels in the 3 groups (ANOVA)

Variable	Group C	Group P	Group PC	<i>p</i> -values
ABL [mm]	0.118 ±0.040	0.718 ±0.242	0.319 ±0.031	C vs P (MD: -0.600) <i>p</i> < 0.05* C vs PC (MD: -0.201) <i>p</i> < 0.05* P vs PC (MD: 0.399) <i>p</i> < 0.05*
IL-1β [pg/mL] in the serum	15.135 ±0.920	92.231 ±1.730	11.248 ±2.180	C vs P (MD: -77.096) <i>p</i> < 0.05* C vs PC (MD: 3.887) <i>p</i> < 0.05* P vs PC (MD: 80.983) <i>p</i> > 0.05
IL-10 [pg/mL] in the serum	1.245 ±0.840	14.742 ±1.780	2.578 ±0.630	C vs P (MD: -13.497) <i>p</i> < 0.05* C vs PC (MD: -1.333) <i>p</i> < 0.05* P vs PC (MD: 12.164) <i>p</i> < 0.05*
TNF-α [pg/mL] in the serum	2.536 ±0.470	16.574 ±0.730	2.988 ±0.340	C vs P (MD: -14.038) <i>p</i> < 0.05* C vs PC (MD: -0.452) <i>p</i> > 0.05 P vs PC (MD: 13.586) <i>p</i> < 0.05*
MDA [μmol/g of protein] in the serum	1.702 ±0.107	2.462 ±0.270	1.805 ±0.070	C vs P (MD: -0.760) <i>p</i> < 0.05* C vs PC (MD: -0.103) <i>p</i> > 0.05 P vs PC (MD: 0.657) <i>p</i> < 0.05*
GSH [μmol/g of protein] in the serum	2.562 ±0.080	2.236 ±0.065	2.851 ±0.219	C vs P (MD: 0.326) <i>p</i> < 0.05* C vs PC (MD: -0.289) <i>p</i> > 0.05 P vs PC (MD: -0.615) <i>p</i> < 0.05*
GSH-Px [IU/g of protein] in the serum	60.361 ±8.962	50.814 ±11.712	80.136 ±5.777	C vs P (MD: 9.547) <i>p</i> < 0.05* C vs PC (MD: -19.775) <i>p</i> > 0.05 P vs PC (MD: -29.322) <i>p</i> < 0.05*
MDA [μmol/g of protein] in the heart	20.341 ±3.014	30.346 ±1.726	20.634 ±2.493	C vs P (MD: -10.005) <i>p</i> > 0.05 C vs PC (MD: -0.293) <i>p</i> > 0.05 P vs PC (MD: 9.712) <i>p</i> > 0.05
GSH [μmol/g of protein] in the heart	6.882 ±1.027	4.205 ±0.647	8.075 ±1.300	C vs P (MD: 2.677) <i>p</i> < 0.05* C vs PC (MD: -1.193) <i>p</i> < 0.05* P vs PC (MD: -3.870) <i>p</i> < 0.05*
GSH-Px [IU/g of protein] in the heart	31.346 ±2.726	29.856 ±2.269	37.075 ±1.318	C vs P (MD: 1.490) <i>p</i> < 0.05* C vs PC (MD: -5.729) <i>p</i> < 0.05* P vs PC (MD: -7.219) <i>p</i> < 0.05*

C – control group; P – periodontitis group; PC – periodontitis + caffeic acid phenethyl ester (CAPE) group; IL – interleukin; TNF-α – tumor necrosis factor-alpha; MDA – malondialdehyde; GSH – glutathione; GSH-Px – glutathione peroxidase; IU – international units; MD – mean difference; * statistically significant (*p* < 0.05). Data presented as mean ± standard deviation (*M* ± *SD*).

Similar to previous studies, the present study found that IL-1 β , IL-10 and TNF- α serum levels were high in periodontitis. In the P group, increased MDA levels and decreased GSH and GSH-Px levels in the serum and heart tissue were found. The administration of CAPE to the PC group not only decreased the levels of MDA in heart tissue, it also increased GSH and GSH-Px levels in heart tissue.


A recent meta-analysis reported that an extra missing tooth due to periodontitis is associated with a 1.5% increment in the risk of heart disease and a 1.5% increment in the risk of stroke.³⁴ Periodontal disease and CVD have nearly the same etiology, such as an increased risk with age, male sex, and history of smoking, diabetes, hypertension, and obesity. The relationship between periodontitis and CVD may be due to chronic inflammation and recurrent oral bacteremia, as inflammation plays a pivotal role in the pathogenesis of atherosclerosis.


Conclusions


To the best of our knowledge, this is the first experimental study to assess the effects of CAPE on ABL, cytokine levels and the oxidative status in the serum and heart tissue in an experimental periodontitis model. Our results suggest that periodontal infection may affect the heart due to increased inflammatory and oxidative reactions. We predicted that CAPE would protect periodontal tissues in periodontitis model rats via anti-inflammatory and antioxidant pathways. Caffeic acid phenethyl ester may induce a good host modulatory response to periodontitis. Given that the administration of CAPE reduced the inflammatory burden and systemic oxidative stress affecting heart tissue in our periodontitis model, these findings suggest that CAPE can protect against the adverse effects of excessive ROS in periodontitis, such as oxidative damage to heart tissue in CVD pathogenesis, etc. Clinical studies further evaluating the relationship between periodontal disease and CVD are needed to confirm these findings.

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Effect of metformin on the behavior of dental pulp stem cells cultured on freeze-dried bone allografts

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Conflict of interest

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Abstract

Background. Considering the complications associated with autogenous bone grafting, the use of freeze-dried bone allograft (FDBA) granules may be considered as an alternative treatment plan.

Objectives. The aim of this study was to evaluate the effect of metformin on both the proliferation and osteogenic capability of dental pulp stem cells (DPSCs) cultured on FDBA granules.

Material and methods. First, a pilot study was conducted only on DPSCs to confirm cellular viability and the osteoinducing effect of 100 µmol/L metformin. Next, the cells were loaded on FDBA granules and treated with and without metformin. Finally, the following analyses were performed: scanning electron microscopy (SEM) (cell attachment); the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (proliferation); and alkaline phosphatase (ALP) activity analysis (osteogenic differentiation).

Results. The SEM images revealed that metformin enhanced the adhesion of DPSCs on FDBA granules. In addition, metformin was shown to increase cell proliferation/viability from day 1 to day 7. Compared to the control, a significant difference was observed after 7 days of treatment. Metformin enhanced the osteogenic capability of FDBA in both standard and osteoinducing conditions. An increase in ALP activity was significant after 7 days of treatment. The positive effect of metformin on differentiation was significant in osteoinducing conditions.

Conclusions. Metformin can be applied as an additional osteoinductive factor in bone regeneration treatment. Moreover, scaffolds with controlled release of metformin can be considered a proper osteoinductive bone substitute that may lessen the complications related to applying allograft scaffolds alone.

Keywords: allograft, dental pulp stem cells, tissue engineering, bone regeneration, metformin

Introduction

Different types of bone materials have been used to treat major orthopedic and maxillofacial bone defects.^{1–3} Although their use produces fewer side effects (e.g., less morbidity, nerve damage, bleeding, infection, and loss of function) in comparison with autogenous bone grafts,^{4,5} they lack essential osteoinductive factors to be utilized as bone-grafting progenitor cells.⁶ However, freeze-dried bone allografts (FDBAs) possess osteoinductive growth factors, such as bone morphogenic proteins.^{4,6} Additionally, they have demonstrated the capability to recruit bone-forming progenitor cells and support them following differentiation.⁶ Despite this capability, they still have less osteogenic capacity than autogenous bone grafts, which leads to slower new bone formation in the defective site.^{7,8} Hence, attempts have been made to find a solution to improve the osteogenic ability of FDBA granules.^{9–11} Unfortunately, they were not effective *in vivo*.^{12–15}

It has been proven that metformin, an insulin-inducing agent, can increase mineralization and osteogenic differentiation. In fact, metformin can activate 5' adenosine monophosphate (AMP)-activated protein kinase (AMPK), which regulates osteoblast differentiation in specific tissues.¹⁶ Several studies have evaluated the effect of metformin, and described its positive effect on the proliferation and osteogenic differentiation of stem cells *in vitro*.^{17–20} The enhancing effect of metformin on new bone formation has also been analyzed in *in vivo* conditions.²¹ It has been shown that the application of metformin helps to prevent the development of periodontitis through its osteoinductive features and its ability to regulate blood glucose.²²

In this study, in order to enhance the osteogenic potential of FDBA materials, metformin, an osteogenic factor, was added to the medium. Human dental pulp stem cells (hDPSCs), a suitable cell source for regenerative treatment,^{23–25} were used. This study evaluated the effect of metformin on the proliferation, osteogenic differentiation and adhesion of DPSCs cultured on FDBA granules.

Material and methods

Ethical permission was obtained from the Ethics Committee of Shahid Beheshti Medical University in Tehran, Iran (Code: IR.SBMU.DRC.REC.1395.324).

Isolation and characterization of human DPSCs

Dental pulp stem cells at passage 3 were purchased from the Dental Research Center of Shahid Beheshti University of Medical Sciences. A high expression of mesenchymal stem cell markers (CD44, CD90, CD73,

and CD105; Abcam, Cambridge, UK) and a low expression of hematopoietic cell markers (CD34 and CD45; Abcam) were characterized through flow cytometry analyses. The multilineal differentiation capacity of the cells was investigated through osteogenic and adipogenic differentiation tests. The cells were cultivated in standard Dulbecco's Modified Eagle's Medium-high glucose (DMEM-HG) with 15% fetal bovine serum (FBS) (Invitrogen, Carlsbad, USA) and 1% penicillin/streptomycin 10,000 U/mL (Life Technologies, Carlsbad, USA) at 37°C in a humidified 5% carbon dioxide environment. When the cultures reached 90% confluence, the cells were detached using 0.25% trypsin plus ethylene diamine tetraacetic acid (EDTA) (Life Technologies), and passaged for the following experiments.

Pilot study

Considering the dose-dependent effect of metformin, 100 µmol/L metformin hydrochloride (Alborz Pharmaceutical Co., Tehran, Iran) was chosen based on a previous study.¹⁹ The positive effect of this concentration of metformin on the proliferation and differentiation of DPSCs was evaluated. In brief, the cells at a density of 10⁴ were seeded in 48-well plates with 100 µmol/L metformin hydrochloride, and either the standard medium was added for the proliferation assay or the osteogenic medium for the differentiation assay. Proliferation was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Aldrich, St. Louis, USA) assay after 1, 3 and 7 days of treatment with or without metformin. Osteogenic differentiation was evaluated using an alkaline phosphatase (ALP) activity kit (Sigma Aldrich) after 3, 7 and 14 days. Similar to the proliferation assay, DPSCs cultured in the osteogenic medium only (DMEM-low glucose (LG), 0.2 mol/L ascorbic acid 2-phosphate, 10⁻⁸ mol/L dexamethasone, and 10 mmol/L β-glycerol phosphate; Life Technologies), without metformin, were used as a control for pairwise comparisons at each time point.

Cell seeding

Particulate cortical/cancellous mineralized 1–2-millimeter FDBA granules (Tissue Regeneration Corporation, Tehran, Iran) were placed in 48-well plates and a cell suspension including 2.5 × 10⁴ DPSCs was loaded into the wells. The plates were incubated at 37°C for 1 h. Then, according to the type of evaluation, 2 kinds of media (standard or osteogenic) were added to the wells. The osteogenic medium included DMEM-LG, 0.2 mol/L ascorbic acid 2-phosphate, 10⁻⁸ mol/L dexamethasone, and 10 mmol/L β-glycerol phosphate (Life Technologies). Next, 100 µmol/L metformin hydrochloride was added to the samples. The study groups and the content of the wells are summarized in Table 1.

Table 1. Study groups

Group	Ingredients	Tests
FDDBA + Met	FDDBA + Met + hDPSCs	SEM, MTT, ALP activity
FDDBA	FDDBA + hDPSCs	SEM, MTT, ALP activity

FDDBA – freeze-dried bone allograft; Met – metformin; hDPSCs – human dental pulp stem cells; SEM – scanning electron microscopy; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ALP – alkaline phosphatase.

Cell adhesion

The adhesion of DPSCs was evaluated with a scanning electron microscope (SEM) (VEGA; Tescan, Brno, Czech Republic) at $\times 1,000$ and $\times 2,000$ magnifications 1 and 7 days after cell seeding. After being rinsed with phosphate-buffered sodium (PBS) (Sigma Aldrich), the cells were fixed for 2 h in 2.5% glutaraldehyde and for 1 h in 1% osmium. The samples were then dehydrated with ascending grades (30%, 50%, 70%, 90%, 95%, and 100%) of ethanol and dried in a desiccator for 24 h. Finally, they were covered with gold and analyzed using SEM imaging.

Cell proliferation

In order to evaluate cell viability, the MTT assay was performed 1, 3 and 7 days after seeding. First, the granules were transferred to new wells to avoid counting the cells adhering to the bottom of the plate. Then, MTT was added to the wells and the plates were incubated at 37°C for 4 h. Next, they were incubated with dimethyl sulfoxide (Carlo Erba Reagents, Milan, Italy) overnight. The supernatants were collected and transferred to 96-well plates, and absorbance was read using a microplate reader (Anthos Labtec Instruments, Salzburg, Austria) at a wavelength of 590 nm.

Cell differentiation

The osteogenic differentiation of the stem cells was evaluated 3, 7 and 14 days after cell seeding using an ALP activity kit. This test was performed on the cells seeded in both the non-inducing and inducing media. The cell-scaffold constructs were rinsed twice with PBS, homogenized in lysis buffer (10 mmol/L Tris-HCl – pH 7.5, 1 mmol/L MgCl_2 and 0.05% Triton X-100) and centrifuged at 12,000 rpm for 10 min at 4°C . The cell lysate was mixed and incubated with the ALP assay reagent at 37°C for 30 min, and the absorbance of the resulting ALP activity was quantified by calculating the optical density values at 405 nm. It must be mentioned that the reaction was stopped using 0.5 N sodium hydroxide.

Statistical analysis

The Mann–Whitney U test was used to compare the MTT values. The Kruskal–Wallis test was performed for the multiple group comparison. Data was analyzed using the PASW Statistics for Windows computer software, v. 18.0 (SPSS Inc., Chicago, USA), at a significance level of 0.05.

Results

Pilot study

The positive effect of metformin on the proliferation and differentiation of DPSCs was evaluated in this phase of the study. The proliferation of DPSCs in the metformin group was higher than that in the control group at each time point, but the difference was significant only at day 1 ($p = 0.00$) (Fig. 1). An increase in the number of cells from day 1 to day 7 demonstrated the biocompatibility of this concentration of metformin ($p = 0.00$). In addition, just as with proliferation, the ALP activity of the metformin group was higher as compared to the control group in the osteogenic medium ($p = 0.00$), but the difference between the groups was not significant in the standard medium (Fig. 2). Moreover, it was shown that the osteogenic medium increased the differentiation of both the control and metformin groups ($p = 0.00$) (Fig. 2).

Cell adhesion

The micro-images of the unloaded scaffolds are depicted in Fig. 3. Freeze-dried bone allograft granules had a lamellar texture and a pore size of 50–500 μm . As can be observed in Fig. 3, SEM confirmed the strong attachment of DPSCs to FDDBA granules in the presence of metformin.

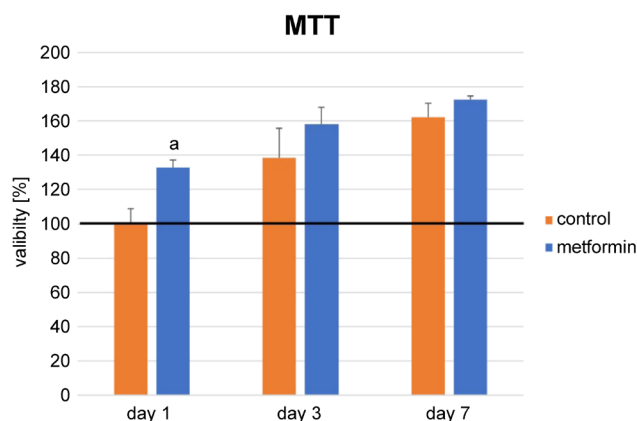


Fig. 1. Comparison of cell viability between the metformin and control groups at different time points

^a statistically significant difference vs control ($p < 0.05$); the error bar represents 95% confidence interval (CI).

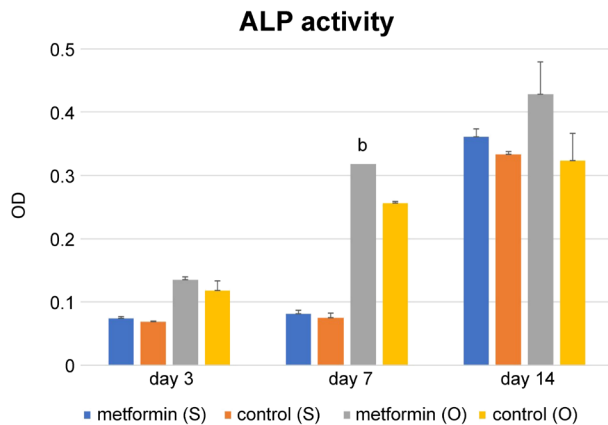


Fig. 2. Comparison of alkaline phosphatase (ALP) activity between the metformin and control groups at different time points

OD – optical density; S – standard medium; O – osteogenic medium; ^b statistically significant difference vs control (O) ($p < 0.05$); the error bar represents 95% CI.

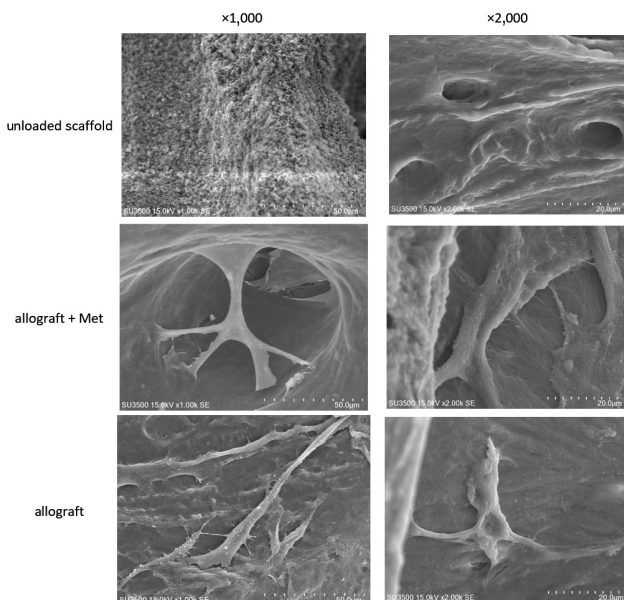


Fig. 3. Scanning electron microscope (SEM) images of the scaffolds 7 days after cell seeding

Cell proliferation

Cell viability increased from day 1 to day 7 in all groups ($p = 0.00$) (Fig. 4). The FDBA + metformin (Met) group showed higher proliferation at each time point. However, the difference was significant only at day 7 following cell seeding ($p = 0.004$).

Cell differentiation

Alkaline phosphatase activity increased in all groups 7 days after cell seeding, in both the inducing and non-inducing media. After 3 days, the FDBA + Met group showed a lower ALP activity in the osteogenic medium, but the difference was not statistically significant.

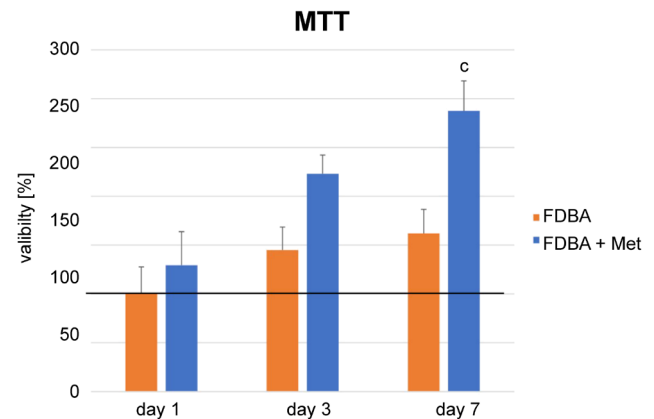


Fig. 4. Comparison of cell viability between the freeze-dried bone allograft (FDBA) + metformin (Met) and FDBA groups at different time points

^c statistically significant difference vs FDBA ($p < 0.05$); the error bar represents 95% CI.

However, after 7 days, the FDBA + Met group showed a significantly higher ALP activity in osteogenic conditions only ($p = 0.01$). Using the osteogenic medium increased ALP activity significantly in both groups ($p = 0.00$), but 14 days following cell seeding, the difference was not significant. In addition, 14 days following cell seeding, there was no significant difference in the ALP activity rate between the FDBA and FDBA + Met groups (Fig. 5). Thus, it was shown that the positive effect of metformin on differentiation was significant in osteoinducing conditions.

Discussion

The success of regeneration treatment depends on providing a suitable environment for the attachment, proliferation and differentiation of stem cells. Proper interactions between stem cells, growth factors and scaffolds are essential to reach this goal. Freeze-dried bone allograft

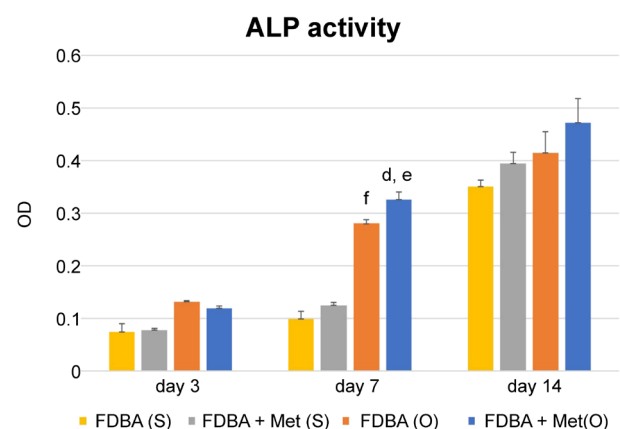


Fig. 5. Comparison of ALP activity between the metformin and control groups at different time points

^d statistically significant difference vs FDBA (O) ($p < 0.05$); ^e statistically significant difference vs FDBA + Met (S) ($p < 0.05$); ^f statistically significant difference vs FDBA (S) ($p < 0.05$); the error bar represents 95% CI.

granules have less osteogenic capacity in comparison with autogenous bone grafts. Thus, several studies have been conducted to enhance the osteogenic ability of these granules. The aim of this study was to evaluate the effect of metformin on the adhesion, proliferation and differentiation of DPSCs cultured on FDBA granules. The results showed that metformin could enhance the adhesion, proliferation and differentiation of DPSCs on FDBA granules in inducing conditions.

Houshmand et al. evaluated the effect of metformin on the proliferation, differentiation and adhesion of DPSCs on macroporous biphasic calcium phosphate granules.¹⁹ They performed a pilot study to determine the proper concentration of metformin. They stated that 100 $\mu\text{mol/L}$ increased the proliferation of DPSCs the most. In addition, they analyzed ALP activity and used the MTT assay to evaluate differentiation and proliferation, respectively.¹⁹ As the present study found, metformin increased proliferation in the presence or absence of the scaffold, and had no significant effect on the differentiation of DPSCs cultured in the non-inducing medium. However, in the inducing medium, metformin increased differentiation.

Several studies have been performed to identify a proper growth factor for enhancing the osteogenic capacity of FDBA granules. Markopoulou et al. noted that recombinant human transforming growth factor-beta 1 (rhTGF- β 1) enhanced the osteogenic differentiation of human periodontal ligament cells (hPDLs) cultured on FDBA granules.¹⁰ In addition, Cenni et al. found that platelet-rich plasma (PRP) had a positive effect in *in vitro* conditions.⁹ However, according to *in vivo* results, PRP had no significant positive effect on the osteogenesis of bone mesenchymal stem cells (BMSCs) cultured on FDBA granules.¹⁵ Thus, our claim that metformin has a positive effect on osteogenesis *in vitro* should be analyzed *in vivo* as well.

Besides *in vitro* studies, several studies have been conducted to improve the osteogenic capacity of FDBA granules *in vivo*. Borie et al. evaluated the effect of autogenous particles and FDBA granules on osteogenesis, using a rabbit calvarial defect model.¹⁴ They found that FDBA granules caused lamellar bone formation after 90 days, while a mixture of autologous particles and FDBA granules caused woven bone formation over the same period of time.¹⁴ In addition, Dallari et al. mentioned in their study that PRP did not enhance the osteogenic capacity of FDBA granules in rabbit trabecular defects.¹⁵ Choukroun et al. found that PRP had no positive effect on the osteogenic capacity of FDBA granules in sinus lift procedures.¹² In addition, Samandari et al. mentioned no positive effect for plasma-rich growth factor on the osteo-inductive ability of FDBA during socket preservation.¹³ Thus, more *in vivo* studies are required to determine a proper material for improving the osteogenic capacity of FDBA granules.

The time- and concentration-dependent effects of metformin on the behavior of stem cells have been proven.²⁶ However, a particular concentration of metformin does not seem to have the same effect on different types of stem cells. For instance, Śmieszek et al. stated that metformin at concentrations of 100 μM and 500 μM did not affect the proliferation of BMSCs significantly, while 10 μM metformin decreased proliferation.²⁶ However, Wang et al. found that 10 μM metformin had no significant effect on the viability of human-induced pluripotent stem cell-derived mesenchymal stem cells (hiPSC-MSCs).²⁰ Zhang et al. mentioned that the 10 $\mu\text{M/L}$ and 50 $\mu\text{M/L}$ concentrations of metformin had no significant effect on the proliferation of periodontal ligament stem cells (PDLSCs), but 100 $\mu\text{M/L}$ metformin increased proliferation.¹⁷ In fact, due to the saturation of ligands, higher concentrations of metformin cannot promote its effects.²⁷ Besides, higher concentrations of metformin may have adverse effects on the energy metabolism of cells, and result in a decrease in proliferation and differentiation.^{17,28}

The exact molecular mechanism of metformin is still unclear. Mu et al. proposed that metformin could enhance differentiation and proliferation through AMPK-mammalian target of rapamycin Complex 2 (AMPK-mTORC2) and Akt-mTOR Complex 1 (Akt-mTORC1) signaling pathways.²⁹ However, Ma et al. stated that metformin increased the osteogenic differentiation of BMSCs by inhibiting glycogen synthase kinase 3 beta (GSK3 β) and Wnt signaling pathways.¹⁸ In addition, they mentioned that AMPK played a crucial role in the abovementioned procedure.¹⁸ Several other studies have mentioned that metformin induces osteogenic differentiation through AMPK and mitogen-activated protein kinase (MAPK) pathways.^{20,21,29,30} Thus, there is still controversy over the intracellular pathways regarding the effect of metformin.

In this study, the effect of metformin on the behavior of DPSCs was evaluated. Several other studies have been conducted to assess the reaction of other types of cells to metformin. The positive effect of metformin on the proliferation and differentiation of murine pre-osteoblasts,²⁹ PDLSCs,³¹ BMSCs,³² and induced pluripotent stem cells²⁰ has been described. In addition, Qin et al. showed that besides osteogenic differentiation, metformin could enhance the odontogenic differentiation of DPSCs.^{33,34}

Considering the limitations of applying autogenous bone grafts, a wide range of natural and synthetic scaffolds has been used in bone regeneration treatment.² However, due to the lower osteogenic capacity of these scaffolds in comparison with autogenous grafts, applying them by themselves has yet to be considered.⁶ The addition of growth factors, one of the fundamental components of tissue engineering besides stem cells and scaffolds, can be deemed a possible method for enhancing the osteoinductivity of bone substitutes. In this study, a significant positive effect of metformin was shown *in vitro*. Therefore, this factor may improve the efficacy of both natural and synthetic scaffolds *in vivo*.

Since this study was performed in vitro, factors related to the recipient features, including the mechanical features of the tissue, possible inflammation, a heterogeneous tissue environment, and interactions between different cell types, were not considered. Therefore, for generalizing the results to in vivo conditions, performing animal and clinical studies is necessary.

Conclusions

The positive effect of metformin on cell proliferation and differentiation, in conjunction with FDDB granules, would be useful for its future application in fabricating FDDB granules with controlled release of this osteoinductive factor for bone tissue engineering. Besides, metformin can be applied in bone regeneration treatment to enhance osteogenesis.

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Three-dimensional accuracy of innovative implant-level impression techniques with plastic snap-on impression copings

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Abstract

Background. Several studies have assessed the accuracy of the indirect snap-on impression technique. However, some impression techniques that utilize plastic snap-on impression copings have not yet been investigated.

Objectives. This study aimed to assess the three-dimensional (3D) accuracy of innovative implant-level impression techniques with plastic impression copings and the splinted open-tray technique with metal impression copings.

Material and methods. This in vitro study used a reference model of the mandible with 4 parallel dental implants. Forty impressions were made with polyether impression material, using the splinted open-tray technique with metal impression copings (SOM group), the non-splinted closed-tray technique with snap-on impression copings (NCS group), the non-splinted open-tray technique with snap-on impression copings (NOS group), or the splinted open-tray technique with snap-on impression copings (SOS group); $n = 10$ per group. Linear discrepancies in the inter-implant distances on the obtained casts were determined in the X, Y and Z axes with the use of a coordinate measuring machine. Subsequently, the 3D accuracy of each impression technique was calculated. Data was analyzed by means of the one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests.

Results. Among different impression techniques, the amount of linear discrepancy was significant only for the Z axis. The SOM, NOS and SOS groups showed less discrepancy in this axis as compared to the NCS group ($p < 0.001$). A significant difference was also noted in 3D discrepancy ($p = 0.022$), with the SOM group showing a significantly higher discrepancy as compared to the SOS group ($p = 0.016$).

Conclusions. The 3D accuracy of the implant-level splinted open-tray impression technique with plastic snap-on impression copings was significantly higher than that of the splinted open-tray technique with metal impression copings.

Keywords: dental implants, dental impression technique, implant-supported dental prosthesis

Introduction

Dental implants are currently a common treatment option for completely or partially edentulous patients.¹ The success rate of dental implant treatment is approx. 97–99%.² Nonetheless, the risk of failure still exists, which is mainly due to incorrect surgical or prosthetic approaches.² Achieving a passive fit is a fundamental goal for implant-supported prostheses, and it is a prerequisite for the preservation of osseointegration.^{3,4} However, it has been suggested that obtaining a prosthesis with an absolute passive fit is impossible in practice.⁵ Evidence shows that, unlike natural teeth, dental implants have highly limited mobility (~10 µm) due to the absence of the periodontal ligament.⁶ This means that any misfit in superstructures can result in applying loads to the implant, causing the accumulation of tension and subsequent complications, including screw loosening, screw fracture, implant fracture, prosthetic framework fracture, veneer fracture, or even osseointegration loss.^{7,8}

Errors in any step of the fabrication of implant-supported restorations can affect the passive fit. However, many of these errors can be avoided by dental clinicians. Precise impression-making and the three-dimensional (3D) simulation of the intraoral implant or abutment positions on a master/working cast are critical steps for the long-term success of prosthetic restorations.^{5,9,10} A number of factors can affect the accuracy of impressions, such as the impression technique,¹¹ the splinting of impression copings,^{12,13} the number of implants,¹⁴ the angulation of the implant or the abutment,¹⁵ the type of impression material,¹⁶ the type of implant connection,¹⁷ and the depth of implant placement.¹⁸

Impressions can be performed at the implant level or the abutment level, and can be made directly (the open-tray or pick-up technique) or indirectly (the closed-tray or repositioning technique). Many studies have evaluated and compared impression techniques in fully or partially edentulous patients.^{5,16,19,20} According to a systematic review, in both completely and partially edentulous patients, the splinted and open-tray impression techniques have a higher accuracy than the non-splinted and closed-tray impression techniques.²¹

At present, the manufacturers of implant systems have introduced various components to enhance impression techniques and increase their accuracy. However, evidence supporting the effectiveness of these components is limited. To minimize the frequency of tightening the abutments, many implant systems have introduced plastic snap-on impression copings for abutment-level impressions,¹⁹ which can also be used for implant-level impressions. Some studies have evaluated the accuracy of these impression copings, reporting conflicting results.^{3,4,11,19,22–27} In some implant systems, plastic impression copings have a unique property that enables the passage of a guide pin through them. Thus, these systems also allow for open-tray impressions,

and can potentially be applied as an alternative to other implant impression techniques. To date, no previous study has assessed the accuracy of this modified impression technique. Thus, the purpose of this study was to compare the 3D accuracy of different innovative implant-level impression techniques, namely the non-splinted closed-tray technique, the non-splinted open-tray technique, and the splinted open-tray technique with plastic snap-on or metal impression copings. The null hypothesis was that no differences in the 3D accuracy of these different impression techniques would be found.

Material and methods

This *in vitro* experimental study evaluated 4 implant-level impression techniques: the splinted open-tray technique with metal impression copings (SOM group); the non-splinted closed-tray technique with snap-on impression copings (NCS group); the non-splinted open-tray technique with snap-on impression copings (NOS group); and the splinted open-tray technique with snap-on impression copings (SOS group). Ten impressions were made with each technique ($n = 10$). The working casts were poured and the inter-implant measurements were made separately on each cast. The discrepancies between the measured values and the actual values measured on the reference model were calculated and statistically analyzed.

For the fabrication of the reference model, several layers of sticky wax were applied on the edentulous ridge of an edentulous model of the mandible to obtain adequate ridge diameter and height for implant placement. Next, it was flaked and auto-polymerizing acrylic resin (Technovit® 4000; Heraeus Kulzer GmbH, Wehrheim, Germany) was used for the fabrication of the model. Four holes, at a distance of 10 mm from each other, parallel to each other and perpendicular to the horizontal plane between the 2 mental foramina were created in the reference model. Bone-level dental implants (Dio® SM-submerged; Dio Corporation, Busan, South Korea) with Torx® internal connections, measuring 4.5 mm in diameter and 12 mm in length, were fixed in place by means of auto-polymerizing acrylic resin (Technovit 4000). A surveyor was used for the parallel insertion of the dental implants.

To ensure the accurate and reproducible positioning of the special tray, 3 stops were created in the land area of the reference model. The transfer copings were tightened onto the implants, and then a primary impression was made with a prefabricated tray, using condensation silicon putty and light material (C-silicone Speedex®; Coltène/Whaledent AG, Altstätten, Switzerland). The primary impression was poured with type IV dental stone (GC Fujirock® EP; GC America Inc., Alsip, USA). A primary cast was fabricated as such for the fabrication of the special tray. Forty special trays ($n = 10$ for each impression technique) were fabricated on the primary cast with

the use of light-polymerized acrylic resin (Megatray™; MEGADENTA Dentalprodukte GmbH, Radebery, Germany). All trays had a thickness of 2 mm and an internal space of 3 mm. The trays were perforated at 1-centimeter intervals. Fifteen minutes prior to impression-making, tray adhesive was applied on the internal surface of the trays and 5 mm around the tray borders.¹⁶

For the SOM technique, the metal open impression copings (regular Torx pick-up impression copings, SIP 4813T; Dio Corporation) were first tightened on the reference model implants with a torque of 10 Ncm. Auto-polymerizing acrylic resin (Pattern Resin™; GC Corporation, Tokyo, Japan) and dental floss were used for the splinted techniques. To ensure the standardization of the shape and amount of the splint material used for each impression, a silicon putty index was made. Next, the acrylic resin was mixed and packed in the index, and the impression copings were attached. After 17 min and ensuring an adequate setting of the acrylic resin, the attachments were separated with a disk. After 24 h, the space was filled with a minimum amount of fresh acrylic resin by means of a brush head and it was allowed to set (Fig. 1). Direct impressions were then made so that the impression material was injected into the tray and around the copings. The tray was then placed over the reference model. An adequate amount of time was allowed for the setting of the impression material, as outlined in the manufacturer's instructions. Next, the guide pins were removed from the implants and the tray, and the splinted impression copings were removed from the reference model. Eventually, the implant analogs were connected to the copings with the help of the guide pins and the impression was poured.

For the other techniques (snap-on), the abutments (Dio SM cemented abutments; Dio Corporation), 4.3 mm diameter and with a 1-millimeter cuff, were attached to the implants with a torque of 10 Ncm, and for making impressions with the abutments, plastic snap-on impression copings (regular impression caps, SASI 4810(II); Dio Corporation) were used. These snap-on copings were uniquely designed to enable the passage of a guide pin through them. In the case of the NCS technique, the plastic

snap-on impression copings were placed on the abutments (Fig. 2) and an indirect impression of the assembly was made. In the NOS and SOS techniques, the plastic snap-on impression copings were used for direct impressions. For this purpose, the abutments, along with the plastic impression copings, were placed on the implants, and then a guide pin was used to tighten the abutment and impression coping assembly (Fig. 3). In the SOS technique, unlike in the NOS technique, splinting was performed, as described for the 1st technique (Fig. 4).



Fig. 2. Non-splinted closed-tray snap-on technique (NCS). The abutments were tightened on the model and the plastic impression copings were placed over them

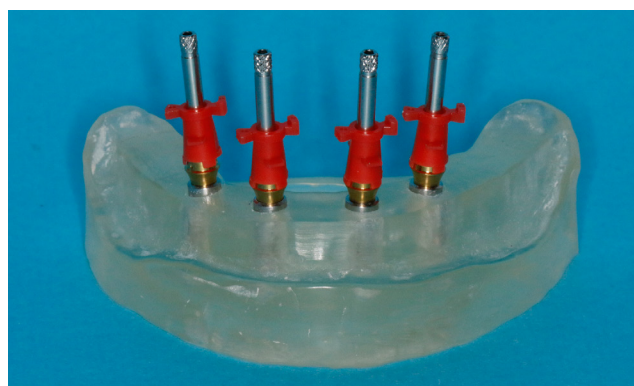


Fig. 3. Non-splinted open-tray snap-on technique (NOS). The abutment–plastic impression coping assembly was tightened to the model with a long guide pin

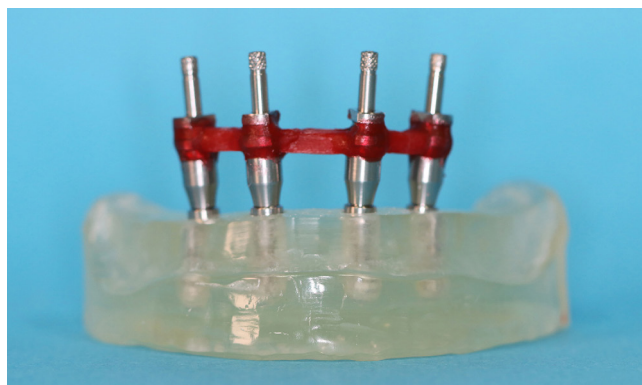


Fig. 1. Splinted open-tray technique with metal impression copings (SOM). The impression copings were splinted with auto-polymerizing acrylic resin



Fig. 4. Splinted open-tray snap-on technique (SOS). The abutment–plastic impression coping assembly was tightened to the model with a long guide pin and splinted with auto-polymerizing acrylic resin

All impressions were made using polyether elastomeric impression material (Impergum™; 3M ESPE, St. Paul, USA) by an experienced technician in a temperature-controlled environment. A 1.5-kilogram metal block was used for standard load application to each tray during the polymerization of the impression material.²² To simulate intraoral conditions, the impressions were set in distilled water at $37 \pm 2^\circ\text{C}$. The impressions were poured after 2–3 h with type IV dental stone (GC Fujirock EP) at a 30 g/7 mL ratio, according to the standard technique.¹⁶ Prior to the removal of the impression from the cast, the casts were allowed to set for 2 h. One technician performed all laboratory procedures and new pieces were used for each impression. To achieve dimensional stability, the casts were stored for 7 days prior to measurements.²⁶

All 40 casts were analyzed in terms of 3D accuracy with a coordinate measuring machine (Miracle Series CMM; Qingdao Leader Metrology Instruments Co., Ltd., Qingdao, China) connected to a computer. After ensuring the calibration of the device, all measurements were made by a researcher blinded to the group allocation of the casts (different impression techniques). The measurements on each cast were performed in triplicate and the mean values were calculated. The measurements were made according to Buzayan et al.²⁸; the implants were numbered 1–4 from left to right. The center of each implant was determined by the coordinate measuring machine probe touching 4 points at the periphery of its external margin. The center of implant #1 was considered as the reference point for all measurements in the X, Y, and Z axes. A hypothetical line passing through the centers of implants #1 and #4 indicated the Y axis. The distance between the center of each implant and that of implant #1 in the X and Y axes, and the vertical distance between the planes of each implant relative to implant #1 were measured to determine the inter-implant distances in the Z axis. The discrepancies between the measured values and the corresponding values on the reference model were calculated (ΔX , ΔY and ΔZ). The mean values of the discrepancies for each implant in each of the X, Y and Z axes were then calculated for each cast. The following formula was used to calculate the 3D discrepancy value for each implant (Equation 1):

$$d = \sqrt{(X_r - X_i)^2 + (Y_r - Y_i)^2 + (Z_r - Z_i)^2} \quad (1)$$

where:

d – 3D discrepancy;

X_r, Y_r, Z_r – inter-implant distances relative to the reference point in the reference model; and

X_i, Y_i, Z_i – inter-implant distances relative to the reference point on the master cast.

Next, the mean spatial position of each cast was calculated. The data was then statistically analyzed.

Statistical analysis

The Shapiro–Wilk test confirmed a normal distribution of the data. Thus, the one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests were used to compare the mean linear discrepancies in each of the X, Y and Z axes, and the 3D discrepancies between different impression techniques. The level of significance was set at 0.05. In data analysis, since deviation in any direction is equally unacceptable, all distortion values were considered 'positive'.^{28,29}

Results

Table 1 shows the means (M) and standard deviations (SD) of the linear discrepancies in each of the X, Y and Z axes, and the 3D discrepancies for different impression techniques. The overall analysis showed significant differences in the 3D discrepancy values ($p = 0.022$) and the linear discrepancy values ($p < 0.001$) in the Z axis between different impression techniques, whereas differences for the X and Y axes were not significant ($p = 0.055$ and $p = 0.080$, respectively). In the Z axis, the discrepancy values in the SOM, NOS and SOS groups were significantly lower as compared to the NCS group ($p < 0.001$). In terms of 3D discrepancy, the SOM group showed the maximum and the SOS group showed the minimum discrepancy, and the difference between these 2 groups was statistically significant ($p = 0.016$).

Discussion

Accurate implant impression-making and the subsequent fabrication of a precise master cast are critical steps for achieving a passive fit and minimizing clinical complications.¹⁶ Thus, many attempts have been made to introduce novel impression techniques. This study evaluated modified impression techniques using plastic snap-on impression copings. The results revealed significant differences in the 3D accuracy of different implant-level impression techniques with plastic snap-on and metal impression copings. Thus, the null hypothesis of the study regarding the absence of a significant difference in the 3D accuracy of master casts fabricated by means of different impression techniques was refuted.

Many factors, i.a., the type of impression material, can affect the accuracy of impression techniques.¹⁶ In this study, polyether impression material was used for all impressions. Polyether impression material has excellent properties, such as a high tear resistance, the favorable reconstruction of details, a high accuracy, and excellent dimensional stability, which make it suitable for dental implants.³ Nonetheless, some studies have reported no differences in accuracy between polyether and polyvinyl siloxane impression material for implant impressions.^{30,31}

Table 1. Linear and three-dimensional (3D) discrepancies [mm] for the 4 impression techniques ($n = 10$)

Impression technique	X axis	Y axis	Z axis	3D
SOM	0.229 ±0.152	0.137 ±0.106	0.017 ±0.017	0.280 ±0.165
NCS	0.164 ±0.115	0.096 ±0.082	0.075 ±0.044	0.221 ±0.119
NOS	0.146 ±0.082	0.096 ±0.087	0.014 ±0.009	0.187 ±0.098
SOS	0.125 ±0.071	0.057 ±0.061	0.023 ±0.014	0.149 ±0.076

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

Although splinting in impression-making is time-consuming, it can transfer the inter-implant relationship to the cast more accurately and prevent the rotation of impression copings.³² Various materials, such as impression plaster, polyether, light-polymerized composite resin, and auto-polymerizing acrylic resin with or without dental floss, are used for splinting; however, auto-polymerizing acrylic resin is most commonly used for this purpose.¹¹ A previous work has shown that the volumetric shrinkage of acrylic resin is 7.9% in the first 24 h, 80% of which occurs in the first 17 min after mixing at room temperature.³³ Thus, it is recommended to fabricate the splint 1 day prior to impression-making.^{6,34} Also, it is recommended to cut the fabricated splint after setting and reattach it again with the same material to minimize volumetric shrinkage.³⁵ In this study, auto-polymerizing acrylic resin and dental floss were used for the splinted techniques. Also, since the shrinkage is proportionate to the amount of resin, the thickness of the splint bar was standardized using a silicon mold.^{11,28}

Many studies have assessed the accuracy of implant impressions with the use of a Vernier caliper, a micrometer, a strain gauge, or a measuring microscope, which only enable two-dimensional (2D) measurements and cannot specify the direction of distortion.^{36–38} Thus, a coordinate measuring machine was used in the current study, as it has a high accuracy and reproducibility, and is less dependent on the operator as compared to other methods.²⁶ Moreover, we used the “relative” distortion analysis suggested by Buzayan et al.,²⁸ which allowed for the inter-implant distances to be measured linearly and three-dimensionally relative to implant #1.

According to the current results, the implant-level splinted open-tray snap-on impression technique was significantly more accurate than the splinted open-tray technique with metal impression copings. As mentioned earlier, no previous study has compared these 2 impression techniques, and thus there is no previous work to compare our results with. However, many studies have compared the accuracy of indirect snap-on and direct impression techniques with metal impression copings. In the current study, the 2 abovementioned impression techniques showed no significant difference in the 3D accuracy of the casts, which is in line with the findings of some previous research.^{3,4,19,24,26} However, some differences exist in the methodology and the techniques employed across these studies.

Nakhaei et al. found that the implant-level snap-on impression technique had similar 3D accuracy to the open-tray technique.⁴ These authors used the non-splinted open-tray technique and a specific implant-level impression coping for the snap-on impression technique.⁴ Similarly, Teo et al. showed that the 3D accuracy of the abutment-level snap-on impression technique was comparable to that of the implant-level non-splinted direct technique when the inter-implant angulation was lower than 15°. ¹⁹ According to the results of Akça and Cehreli, the angular and positional accuracy of the snap-on closed-tray technique with a stock tray and polyvinyl siloxane impression material was similar to that of the non-splinted open-tray technique with metal impression copings and polyether impression material.²⁴ In a study by Wegner et al., the 3D accuracy of the snap-on technique was comparable to that of the non-splinted direct technique.²⁶ Alikhasi et al. evaluated the 3D accuracy of the abutment-level snap-on impression technique and the implant-level open-tray and closed-tray techniques for a three-unit fixed partial denture.³ It was found that the magnitude of displacement in the X, Y and Z axes was similar for those different techniques, while the angular displacement was greater in the snap-on technique.³

Some studies have also reported contradictory results. Walker et al. observed that the 3D accuracy of the indirect impression technique with metal impression copings was higher than that of the impression technique with plastic impression caps at the implant level or the abutment level.²³ Tsagkalidis et al. studied the 3D accuracy of the indirect snap-fit techniques (impression copings along with impression caps) and the splinted and non-splinted direct techniques, and reported that the splinted impression technique provided maximum accuracy, while the snap-fit indirect technique showed minimum accuracy.¹¹ A study by Izadi et al. was among the limited studies that, similar to the current study, used the snap-on technique and the respective abutment for the implant-level impression; however, the authors used the closed-tray technique.²² That study showed that the accuracy of the snap-on technique was lower than that of the non-splinted open-tray technique and similar to that of the closed-tray technique with metal impression copings.²² Fernandez et al. showed that the comparison of impression techniques would yield different results for different implant systems.²⁵ For example, with the use of the Straumann® implant system, metal impression copings yielded a higher accuracy than

the snap-on technique, while with the use of NobelReplace® implant system, no difference was detected between different impression techniques. Thus, impression techniques should be evaluated with regard to different implant systems.²⁵

The current results revealed no significant differences in the 3D accuracy of different snap-on impression techniques. The difference between the open-tray and closed-tray snap-on techniques was not significant, and splinting did not yield a higher accuracy. Several studies have compared the accuracy of the open-tray and closed-tray impression techniques, reporting conflicting results. A systematic review by Kim et al. evaluated relevant articles published in 1990–2013.²⁰ Of the retrieved articles, most supported the open-tray technique, and only one study supported the use of the closed-tray technique.²⁰ Other studies found no significant differences between the 2 techniques. Several studies have also evaluated the effect of splinting on the accuracy of impressions, reporting conflicting results. However, the majority of them noted that the splinting of impression copings yielded superior results.^{5,20,39,40} The current results are in line with studies that found no significant differences in the accuracy of the implant-level splinted and non-splinted impression techniques for a complete dental arch.^{41–43} The discrepancies across studies may be due to differences in the study design, the number of implants, the implant system or the impression material used, splinting, the choice of the implant-level or abutment-level impression, the operator's expertise, the implant angulation, and dental stone expansion.^{11,28} However, it should be noted that no study compared the open-tray and closed-tray or splinted and non-splinted snap-on techniques. This can largely explain the differences in results.

Limitations


One limitation of the current study is the assessment of the accuracy of the impression techniques in the parallel placement of implants in the reference model, which is an ideal situation. Different results may be obtained with the angulated placement of implants, as the effect of the implant angulation on the impression accuracy has been previously confirmed.²⁰ On the other hand, factors such as temperature, moisture and saliva in the oral cavity, which could not be simulated in this in vitro study, might affect the results. Moreover, the reference model used in this study was rigid and made of acrylic resin, different from flexible intraoral soft tissues, which easily undergo distortion upon load application. Due to the different nature of tissue undercuts in the oral cavity in comparison with the in vitro setting, load, the direction of the removal of the impression tray and its subsequent distortion in vitro are different from the clinical setting. Thus, long-term in vivo studies are required to obtain more accurate results.

Conclusions

Within the limitations of this in vitro study, it may be concluded that in a completely edentulous dental arch with 4 parallel dental implants, the 3D accuracy of different snap-on impression techniques (non-splinted closed-tray, non-splinted open-tray and splinted open-tray) is the same. However, the implant-level splinted open-tray impression technique with plastic snap-on impression copings showed a significantly higher accuracy than the splinted open-tray impression technique with metal impression copings.

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Comparative analysis of stress distribution around CFR-PEEK implants and titanium implants with different prosthetic crowns: A finite element analysis

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Abstract

Background. Polyetheretherketone (PEEK) is a new material that was introduced for the fabrication of implants and their superstructure along with other available materials. It is not yet known whether the carbon fiber-reinforced polyetheretherketone (CFR-PEEK) material can be used as an implant and its superstructure in place of titanium (Ti).

Objectives. The study evaluated stress distribution around CFR-PEEK implants and Ti implants with 5 different prosthetic crowns.

Material and methods. A three-dimensional (3D) model of a bone block was created to represent the right maxillary premolar area with a bone-level implant system with 100% osseointegration, using the Ansys Workbench software, v. 15.0. In total, 10 3D finite element analysis (FEA) models were created. The models were divided into 2 groups according to the type of implant: the CFR-PEEK group ($n = 5$); and the Ti group ($n = 5$). Each group was subdivided to imitate 5 different restorative crown materials (PEEK, zirconia, porcelain fused to metal (PFM), metal, and acrylic resin). Each implant model was loaded vertically (200 N) and obliquely (100 N). Stress distribution in the implants, the abutments, the cement layers, and the crowns was evaluated using the von Mises stress analysis. Maximum and minimum principal stress analyses were used to determine the stress generated in the bone.

Results. The CFR-PEEK implants bore more stress in vertical and oblique loading as compared to the Ti implants. The stress generated in the bone with the CFR-PEEK implants was similar to that generated with the Ti implants under vertical loading. Under oblique loading, less stress was transferred to the bone with the CFR-PEEK implants as compared to the Ti implants, showing better adaptation of the CFR-PEEK implants to lateral stress.

Conclusions. In this FEA study, the amount of stress generated within the bone in the case of the CFR-PEEK implants with different restorative crowns was smaller in comparison with the Ti implants in oblique loading. This could help reduce lateral stress on implants as well as crestal bone loss.

Keywords: dental implant, finite element analysis, polyetheretherketone, titanium, zirconium oxide

Introduction

Dental implant-supported prostheses are becoming the preferred treatment option in dentistry to replace missing teeth due to their long-term survivability and proven advantages.^{1,2} The pattern of stress distribution during mastication differs in implants, as the forces are directly transferred to the adjacent surrounding bone, which plays a vital role in the success of implants.^{3,4} The lack of the periodontal ligament in dental implants causes decreased proprioception at the implant–bone interface. This decreased resilience results in increased forces, which often leads to implant failure and chipping of dental porcelain.^{5,6}

Titanium (Ti) is the material of choice for dental implants; it was introduced by Brånemark in 1978.⁷ However, certain drawbacks with regard to the use of Ti have been reported in the literature, such as its tendency to cause hypersensitivity and allergic reactions.^{8–10} Another drawback is that its modulus of elasticity differs from that of the surrounding bone. This causes stress concentration at the implant–bone interface during load transfer, resulting in peri-implant bone loss.¹¹ Titanium also evokes scattering rays when it is in the field of radiation, which is harmful to tissues.¹²

In the last few years, ceramics, mainly yttria-stabilized tetragonal zirconia polycrystalline, have been used as an alternative to Ti implants. They have favorable properties, such as translucency and white color that simulate natural teeth. However, the promotion of ceramic implants to satisfy increasing esthetic demands is fraught with compromise due to the brittle nature of zirconia materials. This significant susceptibility to surface defects can increase stress concentration at these sites.^{13,14}

More recently, polyetheretherketone (PEEK) has become a source of research interest and is being tested for use as an implant material.¹⁵ Polyetheretherketone is a synthetic, polymeric, organic material, developed in 1978, that is characterized by good chemical resistance, good mechanical properties and biocompatibility. Polyetheretherketone is compatible with modern imaging technologies. It is a tooth-colored material that has recently been used as a dental implant material when esthetics is a major concern. Polyetheretherketone is being used for the implant superstructure, the abutment, implant fixture, and implant-supported hybrid prostheses. The Young's modulus of the PEEK material in its pure form is 3.6 GPa, of carbon fiber-reinforced PEEK (CFR-PEEK) around 18 GPa and of glass fiber-reinforced PEEK 12 GPa.^{16,17} The CFR-PEEK material is a biologically inert material. Studies on cytotoxicity, mutagenicity, carcinogenicity, and immune system impairment have found no evidence that it has any harmful effects.^{18–20}

The success of an implant depends on the type of material used for the implant superstructure. Many materials are being used today for implant prostheses, such as porcelain fused to metal (PFM) crowns, all-ceramic

crowns, full-cast crowns, and acrylic resin crowns, with each having its own limitations.²¹ The Young's modulus of CFR-PEEK is close to that of the cortical bone; hence, it exhibits less stress shielding than Ti. However, not much is known about the use of CFR-PEEK as a dental implant and its superstructure.^{22,23} Thus, this study attempted to use the finite element analysis (FEA) to determine the stress generated by this new material on the implant and its adjacent surrounding bone.

The aim of this study was to use FEA to evaluate stress distribution around CFR-PEEK implants and Ti implants with 5 types of prosthetic crowns (PEEK, zirconia, PFM, metal, and acrylic resin) under vertical and oblique loading. The null hypothesis was that there would be no differences in the distribution of stress around CFR-PEEK and Ti implants with different prosthetic crowns.

Material and methods

This experimental study was conducted in the principal author's institution in collaboration with CADD Solutions, Vijayawada, India. The FEA model was generated carefully so that it resembled the real object to ensure an effective and accurate analysis.²⁴

Model generation

An edentulous maxilla was scanned using a dental volumetric computed tomography (CT) device (the HDI 100 Series 3D scanner with the FlexScan3D software; LMI Technologies, Burnaby, Canada). A model was generated to simulate the right maxillary premolar zone with a cancellous bone thickness of 2 cm, surrounded by a 1.5-millimeter-thick cortical bone. The bones were given different colors so that they could be easily identified. The properties of the components generated in the model were kept isotropic, linear and homogeneous.⁵ Movement in the X, Y and Z planes of the bone surface was prevented by keeping the boundary conditions fixed at the alveolar bone level.²⁵ The effect of the gingiva on the implant during loading seemed to be negligible, so it was not modeled in the generated model.^{5,26}

A Ti implant (Ø 4.1 mm × 10 mm) (Straumann® Bone Level Implants; Straumann USA, Andover, USA) with a Ti abutment (Ø 4.5 mm × 4 mm) (Straumann CrossFit® Abutments; Straumann USA) and a Ti screw was scanned with the HDI 100 series 3D scanner. The Standard Tessellation Language (STL) data obtained for each scanned component was transferred into 3D modeling software (Ansys Workbench, v. 15.0; Ansys Inc., Canonsburg, USA). The 3D model of the implant was considered to be 100% osseointegrated in the bone.^{5,22}

In total, 10 3D FEA models were created. The FEA models were divided into 2 main groups based on the type of implant – the CFR-PEEK group implants ($n = 5$) were

given the properties of CFR-PEEK implants and abutments, while the Ti group implants ($n = 5$) were given the properties of Ti implants and abutments. The abutment was placed over the implant in proper adaptation, followed by modeling of the restorative crowns. Each group was subdivided to imitate 5 restorative crown materials (PEEK, zirconia, PFM, metal, and acrylic resin). The restorative PFM crown was modeled with a metal thickness of the coping of 0.5 mm and a porcelain thickness of at least 1.5 mm in the occlusal area of the functional cusp. Similar dimensions of the PEEK crown with a PEEK thickness of the coping of 0.5 mm, layered with a micro-hybrid composite (Ceramage®; Shofu, Inc., Kyoto, Japan) thickness of at least 1.5 mm in the occlusal area of the functional cusp were modeled. The crown thickness for zirconia and acrylic resin was 2 mm, while it was 1.5 mm for the metal crown in the occlusal area of the functional cusp.²⁷ A 30-micrometer-thick layer of dual-polymerized resin cement (Panavia™ F 2.0; Kuraray Europe, Hattersheim am Main, Germany) was also created between the crown and the abutment to exactly simulate clinical conditions.^{5,28}

Setting material properties

The Young's modulus and Poisson's ratio of the various materials were obtained from the published research and installed into the software (Table 1).^{29–35}

Load and constraints

All 10 models were tested in terms of stress distribution with 200 N of vertical load applied to the central fossa and 100 N of oblique load at an angle of 30° applied to the buccal incline of the palatal cusp (Fig. 1A).^{5,36}

Table 1. Young's modulus and Poisson's ratio of each material

Material/Structure	Young's modulus [GPa]	Poisson's ratio
Cortical bone	13.70 ²⁹	0.30 ²⁹
Cancellous bone	1.37 ²⁹	0.30 ²⁹
Ti implant, abutment, screw	110.00 ²⁹	0.35 ²⁹
CFR-PEEK implant, abutment	150.00 ³⁰	0.39*
Porcelain	69.00 ³¹	0.30 ³¹
Zirconia	210.00 ³²	0.30 ³²
Composite	10.70*	0.30*
Acrylic resin	3.00 ³³	0.30 ³³
PEEK	4.10*	0.40*
Ni-Cr	203.60 ³⁴	0.30 ³⁴
Co-Cr	218.00 ²⁹	0.33 ²⁹
Dual-polymerized resin cement	18.60 ³⁵	0.28 ³⁵

Ti – titanium; CFR-PEEK – carbon fiber-reinforced polyetheretherketone; PEEK – polyetheretherketone; Ni – nickel; Cr – chromium; Co – cobalt; * values provided by the manufacturer.

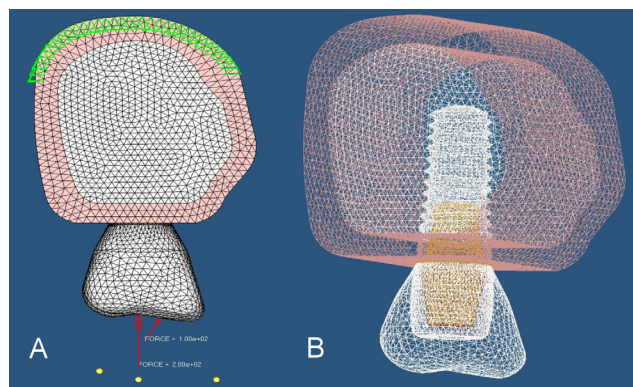


Fig. 1. A – model showing 200 N of vertical load applied to the central fossa and 100 N of oblique load at an angle of 30° applied to the buccal incline of the palatal cusp; B – mesh-generated model

Meshing and contact characteristics

The mesh refinement level was limited by converting the mesh with a controlled and connected element corresponding to each structure (Fig. 1B). Meshing was applied to divide the model into nodes and elements, with 233,750 nodes and 167,348 elements generated in this study.³⁷ Stress distribution in the implants, the abutments, the cement layers, and the restorative crowns was evaluated using the von Mises stress analysis. Maximum and minimum principal stress analyses were used to determine the stress generated in the bone. The von Mises stress analysis was used to determine the effect of the loading forces on the implant and the restorative structures. The generated stress was measured in megapascals (MPa); it provided information about the point where the elastic boundary was exceeded. The comparison among the different models was performed with the help of a color scale representing the levels of the generated stress in different colors.

Results

The forces were applied in 2 directions in each model. The values of von Mises stress as well as of maximum and minimum stress were evaluated for each model; they are presented in Tables 2–6.

The maximum and minimum principal stress values within the bone for the CFR-PEEK and Ti implants with different restorative crowns are shown in Table 2. In vertical loading, the maximum principal stress generated in the bone with the CFR-PEEK implants and the PEEK crown (5.888 MPa), the zirconia crown (5.889 MPa), the PFM crown (5.888 MPa), the metal crown (5.889 MPa), and the acrylic resin crown (5.888 MPa) was slightly greater as compared to the Ti implants and the PEEK crown (5.645 MPa), the zirconia crown (5.649 MPa), the PFM crown (5.645 MPa), the metal crown (5.649 MPa), and the acrylic resin crown (5.648 MPa). Under oblique loading, there was less stress generated in the bone

Table 2. Minimum and maximum principal stress values [MPa] observed in the bone under vertical and oblique loading

Crown	Vertical loading				Oblique loading			
	CFR-PEEK group		Ti group		CFR-PEEK group		Ti group	
	min	max	min	max	min	max	min	max
PEEK	0.654	5.888	0.627	5.645	0.928	8.357	1.039	9.354
Zirconia	0.654	5.889	0.627	5.649	0.922	8.301	1.031	9.282
PFM	0.654	5.888	0.627	5.645	0.925	8.333	1.037	9.330
Metal	0.654	5.889	0.627	5.649	0.922	8.300	1.031	9.282
Acrylic resin	0.654	5.888	0.627	5.648	0.924	8.323	1.034	9.309

PFM – porcelain fused to metal.

for the CFR-PEEK implants with the the PEEK crown (8.357 MPa), the zirconia crown (8.301 MPa), the PFM crown (8.333 MPa), the metal crown (8.300 MPa), and the acrylic resin crown (8.323 MPa) as compared to the Ti implants with the PEEK crown (9.354 MPa), the zirconia crown (9.282 MPa), the PFM crown (9.330 MPa), the metal crown (9.282 MPa), and the acrylic resin crown (9.309 MPa). The CFR-PEEK implant with the PEEK crown showed similar stress in vertical loading (Fig. 2A) and less stress in oblique loading (Fig. 2B) when compared with the Ti implant with the the PEEK crown (Fig. 2C and Fig. 2D). The stress was more concentrated at the crest region near the implant–abutment connection area in oblique loading for both

implant groups, but it was less concentrated in the CFR-PEEK implant group (Fig. 2B).

The von Mises stress values within the implant for the CFR-PEEK and Ti implants with different restorative crowns are listed in Table 3. Under vertical and oblique loading, the stress generated in the implant was greater in the CFR-PEEK implant group with different restorative crowns as compared to the Ti implant group. Under vertical loading, the stress generated in the CFR-PEEK implants in comparison with the Ti implants with different restorative crowns was as follows: the PEEK crown – 221.859 MPa vs 195.693 MPa; the zirconia crown – 221.881 MPa vs 195.072 MPa; the PFM crown – 221.867 MPa vs 195.703 MPa; the metal crown – 221.881 MPa vs 195.072 MPa; and the acrylic resin crown – 221.861 MPa vs 195.044 MPa. Under oblique loading, the stress generated in the CFR-PEEK implants in comparison with the Ti implants with different restorative crowns was as follows: the PEEK crown – 291.150 MPa vs 240.785 MPa; the zirconia crown – 291.098 MPa vs 240.433 MPa; the PFM crown – 291.090 MPa vs 240.718 MPa; the metal crown – 291.096 MPa vs 240.430 MPa; and the acrylic resin crown – 291.164 MPa vs 240.506 MPa. The CFR-PEEK implant with the PEEK crown showed more stress in vertical (Fig. 3A) and oblique loading (Fig. 3B) when compared with the Ti implant with the PEEK crown (Fig. 3C and Fig. 3D). Although in oblique loading, the stress was more concentrated at the crest and body of the implants in both implant groups, the result showed that the maximum stress generated was taken up by the CFR-PEEK implant in a better way as compared to the Ti implant (Fig. 3B).

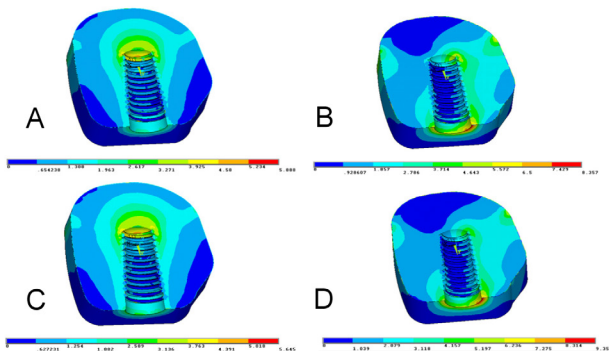


Fig. 2. A – stress generated in the bone in the model with the CFR-PEEK implant–abutment and the PEEK crown in vertical loading; B – stress generated in the bone in the model with the CFR-PEEK implant–abutment and the PEEK crown in oblique loading; C – stress generated in the bone in the model with the Ti implant–abutment and the PEEK crown in vertical loading; D – stress generated in the bone in the model with the Ti implant–abutment and the PEEK crown in oblique loading

Table 3. von Mises stress values [MPa] observed under vertical and oblique loading within the implant

Crown	Vertical loading		Oblique loading	
	CFR-PEEK group	Ti group	CFR-PEEK group	Ti group
PEEK	221.859	195.693	291.150	240.785
Zirconia	221.881	195.072	291.098	240.433
PFM	221.867	195.703	291.090	240.718
Metal	221.881	195.072	291.096	240.430
Acrylic resin	221.861	195.044	291.164	240.506

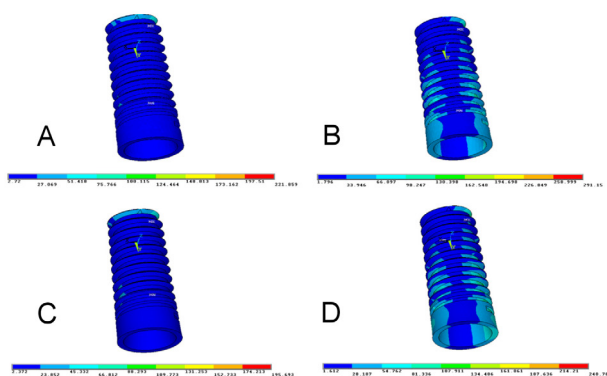


Fig. 3. A – stress generated within the implant in the model with the CFR-PEEK implant–abutment and the PEEK crown in vertical loading; B – stress generated within the implant in the model with the CFR-PEEK implant–abutment and the PEEK crown in oblique loading; C – stress generated within the implant in the model with the Ti implant–abutment and the PEEK crown in vertical loading; D – stress generated within the implant in the model with the Ti implant–abutment and the PEEK crown in oblique loading

The von Mises stress values within the CFR-PEEK and Ti abutments with different restorative crowns are shown in Table 4. The stress generated in the abutment in vertical (84.572 MPa) (Fig. 4A) and oblique loading (101.613 MPa) (Fig. 4B) in the CFR-PEEK abutment with the PEEK crown was slightly greater than in the Ti abutment with the PEEK crown (77.933 MPa and 99.187 MPa, respectively) (Fig. 4C and Fig. 4D). The stress generated in the CFR-PEEK abutments with the zirconia, PFM and metal crowns was lower under both vertical and oblique loading in comparison with similar crowns in the Ti implant group. The stress generated in the CFR-PEEK abutment with the acrylic resin crown was higher under both vertical and oblique loading in comparison with the same crown in the Ti implant group (Table 4). Under vertical and oblique loading, stress concentration was higher at the implant–abutment connection area.

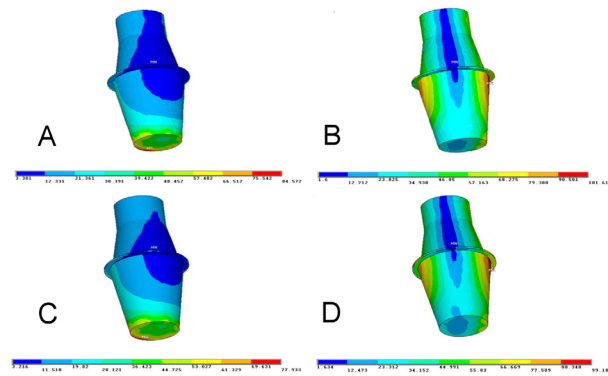


Fig. 4. A – stress generated in the CFR-PEEK abutment with the PEEK crown in vertical loading; B – stress generated in the CFR-PEEK abutment with the PEEK crown in oblique loading; C – stress generated in the Ti abutment with the PEEK crown in vertical loading; D – stress generated in the Ti abutment with the PEEK crown in oblique loading

The von Mises stress values within the cement layer of the different restorative crowns cemented to the CFR-PEEK and Ti abutments are shown in Table 5. Among all of the restorative crowns, the highest stress generated in the cement layer for both types of loading (vertical and oblique) was with the acrylic resin crown. The stress generated in the cement layer in vertical (46.154 MPa) (Fig. 5A) and oblique loading (49.750 MPa) (Fig. 5B) in the CFR-PEEK abutment with the PEEK crown was slightly greater as compared to the Ti abutment with the PEEK crown (44.160 MPa and 46.006 MPa, respectively) (Fig. 5C and Fig. 5D). The amount of stress generated in the cement layer with the CFR-PEEK abutments and the zirconia, PFM and metal crowns was smaller in oblique loading when compared with similar crowns in the Ti implant group (Table 5). The stress generated in vertical loading in both groups was more concentrated toward the occlusal aspect of the

Table 4. von Mises stress values [MPa] observed under vertical and oblique loading within the abutment

Crown	Vertical loading		Oblique loading	
	CFR-PEEK group	Ti group	CFR-PEEK group	Ti group
PEEK	84.572	77.933	101.613	99.187
Zirconia	30.169	35.627	88.867	93.797
PFM	32.304	38.824	90.307	105.947
Metal	33.846	39.136	89.759	102.354
Acrylic resin	109.180	103.525	120.672	111.805

Table 5. von Mises stress values [MPa] observed under vertical and oblique loading within the cement layer

Crown	Vertical loading		Oblique loading	
	CFR-PEEK group	Ti group	CFR-PEEK group	Ti group
PEEK	46.154	44.160	49.750	46.006
Zirconia	8.327	8.929	16.300	18.581
PFM	9.439	8.745	16.420	18.785
Metal	8.295	9.062	16.373	18.835
Acrylic resin	59.870	59.212	69.521	67.610

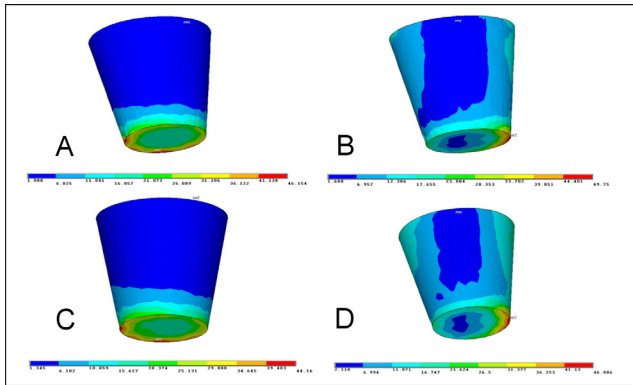


Fig. 5. A – stress generated in the cement layer of the model with the CFR-PEEK abutment and the PEEK crown in vertical loading; B – stress generated in the cement layer of the model with the CFR-PEEK abutment and the PEEK crown in oblique loading; C – stress generated in the cement layer of the model with the Ti abutment and the PEEK crown in vertical loading; D – stress generated in the cement layer of the model with the Ti abutment and the PEEK crown in oblique loading

cement layer, whereas in oblique loading, the stress was more concentrated toward the implant–abutment connection area.

The von Mises stress values within the different crowns cemented to the CFR-PEEK and Ti abutments are presented in Table 6. Under vertical and oblique loading, the stress generated in all of the crowns cemented to the CFR-PEEK abutment was similar to that generated in the crowns cemented to the Ti abutments. The stress generated in the PEEK crown cemented to the CFR-PEEK abutment (Fig. 6A and Fig. 6B) was observed to be similar to that generated in the PEEK crown cemented to the Ti abutment (Fig. 6C and Fig. 6D). Under vertical loading, the stress was more concentrated in the central fossa region of the crowns. Under oblique loading, the stress was more concentrated on the buccal inclines of the palatal cusps and at the margins of the crowns in both groups.

Discussion

This study analyzed the null hypothesis that no difference would be found in stress distribution around CFR-PEEK implants and Ti implants with 5 different prosthetic crowns. Based on the results of this study, the null hypothesis was rejected.

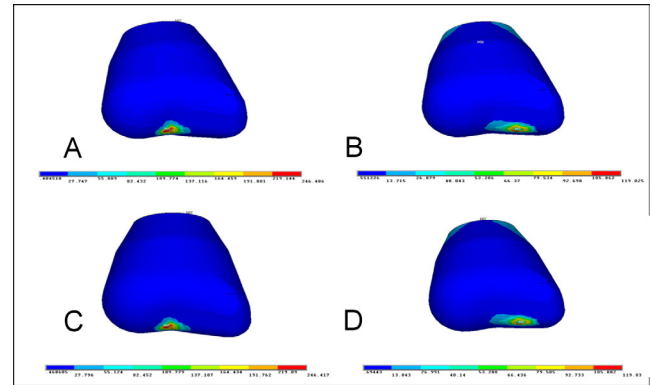


Fig. 6. A – stress generated in the PEEK crown cemented to the CFR-PEEK abutment in vertical loading; B – stress generated in the PEEK crown cemented to the CFR-PEEK abutment in oblique loading; C – stress generated in the PEEK crown cemented to the Ti abutment in vertical loading; D – stress generated in the PEEK crown cemented to the Ti abutment in oblique loading

The finite element analysis is a suitable scientific method for assessing biomechanical behavior in complex configurations. The FEA models can be two-dimensional (2D) or 3D. In this study, 3D models were used to achieve true-to-life results.³⁸ The failure of the material may occur if the von Mises stress values are greater than 550 MPa, which is the yield strength of Ti.³⁹ In this study, all the FEA models of the implants in both subgroups had von Mises stress values well below 550 MPa. Under oblique loading, the highest von Mises stress value was obtained with the CFR-PEEK implant (291 MPa).

Stress dissemination in the implant components, the peripheral bone and the restorative crowns was evaluated under vertical (200 N) and oblique loading (100 N). Continuous concentration of masticatory forces at a point on a dental implant for a long time may lead to implant failure. Taking that into consideration, in this study, the vertical load was applied to the central fossa so that it could be distributed to the palatal and buccal cusps, while the oblique load was applied to the functional palatal cusp.⁴⁰

In this study, CFR-PEEK was used based on a growing interest in its application in implant dentistry due to its adaptability, affinity with present-day imaging technologies, outstanding mechanical properties, and biological acceptability.⁴¹ In this study, under vertical and oblique loading, the CFR-PEEK and Ti implant groups transferred forces in a homogeneous fashion with all restor-

Table 6. von Mises stress values [MPa] observed under vertical and oblique loading within the crown

Crown	Vertical loading		Oblique loading	
	CFR-PEEK group	Ti group	CFR-PEEK group	Ti group
PEEK	246.486	246.417	119.025	119.030
Zirconia	257.298	257.169	123.861	123.864
PFM	229.884	229.772	111.470	111.472
Metal	245.768	245.637	119.019	119.022
Acrylic resin	229.909	229.887	111.517	111.520

ative crowns. Stress in both the CFR-PEEK and Ti implant groups was more concentrated at the implant–abutment connection area, which is consistent with other studies, and this was due to the rigid connection between the implant and the bone (Fig. 2B and Fig. 2D).^{5,16} Due to its increased modulus of elasticity in comparison with the spongy bone, the cortical bone is stronger and more resistant to deformation; the stress values in the prostheses and the peripheral bone increase in oblique loading.⁴⁰ A similar result was obtained in the present study with both the CFR-PEEK and Ti implants (Fig. 2B and Fig. 2D).

Sarot et al. compared the stress distributed by 30% CFR-PEEK and Ti and found that a dental implant with endless carbon fibers can decrease elastic deformation and help decrease the stress peaks at the implant–bone interface.¹⁵ Bataineh and Al Janaideh⁴¹ found that the substitution of a PEEK implant for a Ti implant does not provide any advantages in regard to stress distribution to the peri-implant bone.⁴¹ This finding is contradictory to the present study, in which there was less stress generated in the bone by the CFR-PEEK implants under oblique loading as compared to Ti implants. The reason for this difference in findings may be that Endolign[®] CFR-PEEK was used in the present study. Endolign is a CFR-PEEK material that has 60% endless carbon fibers of parallel orientation. The modulus of elasticity of this material, as provided by manufacturers, is 150 GPa, which is higher than that of Ti (110 GPa) and 30% CFR-PEEK (18 GPa). This provides stability with better stress distribution.³⁰ A similar result was obtained by Schwitalla et al. in their study which compared the biomechanical behavior of Ti, powder-filled PEEK and Endolign as implant materials.³⁰ Powder-filled PEEK showed higher von Mises stress values with maximum deformation, while Ti and Endolign showed similar stress distribution.³⁰

Researchers have assessed the outcome of using various prosthesis materials on stress distribution in the peripheral bone and implants, and suggested that a change in the prosthesis material brought no considerable difference in stress patterns.^{42–45} This corresponds with the present study, with different occlusal materials generating similar stress in the implants. The PEEK crown in the present study generated similar stress with both the CFR-PEEK and Ti abutments.

Tekin et al. assessed the stress created within the abutment and suggested that there was more stress when changing the abutment material than when changing the crown material.¹⁶ Kaleli et al. found that customized zirconia abutments had higher stress values as compared to customized PEEK abutments.⁵ Customized PEEK abutments had a 60 times lower modulus of elasticity than customized zirconia, and demonstrated less stress in the abutment and more stress in the crown.⁴⁶ A similar result was obtained in the present study, in which there was less stress within the abutment and bone, and more stress in the crowns in the CFR-PEEK implant–abutment group.

This result may be due to the use of the CFR-PEEK material, which has a higher modulus of elasticity than Ti, which reduces stress in the bone while having more stress within the CFR-PEEK implant.

In a FEA study by Ahmed et al., Ti implants–abutments under vertical loading generated high stress in the abutment and less stress in the bone.⁴⁷ Stress distribution on the occlusal aspect was similar among different occlusal materials. The model with the porcelain crown received the highest von Mises stress value (345.390 MPa), while the model with the PEEK crown received the lowest von Mises stress value (313.094 MPa).⁴⁷ In the present study, the opposite result was obtained; the model with the PEEK crown had more stress as compared to the PFM crown. The zirconia crown model received the highest von Mises stress value, while the PEEK crown model received a lower von Mises stress value. The reason behind this difference may be due to the modulus of elasticity of zirconia, which made this material more impervious to distortion.

Tekin et al. found less stress with the use of the PEEK abutment with the PFM crown under oblique loading.¹⁶ A similar result was found in the present study, with the CFR-PEEK implant–abutment with different restorative crowns. The reason may be that in this case, the abutment and implant were made of the CFR-PEEK material, which has a high modulus of elasticity (150 GPa). In accordance with the energy dissipation theory, when the force applied to the implant-retained crown dissipates through the implant, as a result of elastic deformation, minimal energy is kept by the implant due to small alterations in the energy conservation feature of rigid implants.⁴⁸

In the study by Tekin et al., it was observed that maximum stress was concentrated on the margins of the crown under oblique loading.¹⁶ Groups with Ti abutments had less stress in the crown as compared to groups with PEEK abutments.¹⁶ The present study showed the opposite result; both the CFR-PEEK and Ti implant groups with different crowns had similar von Mises stress values, and stress was concentrated on the margins of the crowns. The exception was the acrylic resin crowns, which had stress concentrated in the central fossa region.

The stress result of PEEK as a crown material was assessed in this study, and it has shown promising results as a crown material over both the CFR-PEEK and Ti abutments. Carbon fiber-reinforced polyetheretherketone showed good results when used as an abutment and implant, and the results were similar to the Ti implant and abutment with different restorative crowns.


Conclusions


In this FEA study, the amount of stress generated within the bone in the case of the CFR-PEEK implants with different restorative crowns was smaller in comparison with the Ti implants in oblique loading. This could be


beneficial in terms of reducing lateral stress on implants and could help reduce crestal bone loss. The CFR-PEEK material may emerge as an alternative implant material in the near future and will certainly be beneficial for patients with hypersensitivity and allergic reactions to Ti. Further in vivo research should be conducted with PEEK crowns over CFR-PEEK implants–abutments to assess the osseointegration of this material and how this property can be further improved.

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Effect of staining and bleaching on the microhardness, surface roughness and color of different composite resins

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Conflict of interest

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Abstract

Background. There are studies that examined the effect of staining on the surface properties of composite resins, using different solutions and bleaching applications. However, the effect of both staining and bleaching on the same composite specimens is an issue that needs to be investigated.

Objectives. The aim of this study was to investigate the surface microhardness, roughness and color changes (ΔE) of 2 different composite resins after staining and bleaching.

Material and methods. A microhybrid and a nanohybrid composite were used in the study. One hundred and fifty specimens were prepared from each composite. The specimens were divided into 5 groups and stained for 30 days with tea, coffee, cola, red wine, or distilled water (control). Subsequently, each group was divided into 3 subgroups. Each subgroup received a 14-day application of Opalescence™ Boost, Opalescence PF or VivaStyle® Paint On Plus bleaching materials. The color as well as surface microhardness and roughness of all specimens were determined at baseline, after staining and after bleaching. Data was analyzed using the repeated-measures analysis of variance (ANOVA) and the Bonferroni method.

Results. A statistically significant decrease was observed in the surface microhardness of the microhybrid composite specimens after bleaching ($p < 0.05$). The highest ΔE values were observed in the red wine groups for both composite resins.

Conclusions. Staining and bleaching may affect surface properties and color, depending on the type, filler and matrix content of the composite resin.

Keywords: surface roughness, microhardness, bleaching, composite resin, color change

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Introduction

Various kinds of cosmetic treatment are among the most important procedures in modern dentistry. The production of tooth-colored restorative materials, the development of bleaching materials as well as heightened patient expectations have increased the application of cosmetic dental treatment, and thus the clinical use of composite resins.¹ Microhybrid composites are successfully used in anterior and posterior teeth due to their physical and mechanical properties, while nanohybrid composites, produced in recent years, provide clinical success with their attractive appearance and durability.²

The success of composite resin restorations depends on the mechanical and physical properties of the restoration, such as durability, hardness, abrasion resistance, and surface smoothness, the prevention of secondary caries, microleakage and plaque deposition, the appearance of the restoration, and patient satisfaction.³ The hardness of a material is defined as its resistance to surface indentation or abrasion, and is associated with strength and elastic limit. The resistance of a material to abrasion and scratching increases in line with its surface microhardness, while the risk of deformation decreases.⁴ In order to ensure successful restoration and occlusion, composite resins should possess surface hardness and abrasion resistance as close as possible to natural tooth surfaces.

The surface roughness of a composite resin restoration is an important characteristic that affects plaque deposition as well as the water absorption and cosmetic properties of the restoration. Rough surfaces may cause coloring materials to accumulate on the restoration surface, resulting in cosmetic problems.⁵ The colored substances released from commonly consumed beverages, such as tea, coffee or cola, may accumulate on the surface of the composite resin restoration and the teeth, and result in discoloration. Such beverages can also lead to increased discoloration of the composite resin by decreasing its surface hardness and smoothness.⁶ In addition to these extrinsic factors, intrinsic factors, like the structural properties of the resin, and photo-initiators, like camphorquinone, may cause discoloration in composite resins. The matrix structure, the monomer type, and the type and amount of filler may also affect the color and surface properties of the resin. Color measurement may be performed digitally, and the Munsell color system or the International Commission on Illumination (Commission internationale de l'éclairage – CIE) color scale are frequently employed for this purpose.⁷

Bleaching materials used to remove the coloration caused by intrinsic and extrinsic factors are generally divided into 2 types – office or home – depending on their use. These materials, which have similar mechanisms of action, react with and break down the organic pigment molecules responsible for tooth discoloration.⁸ The most commonly used active ingredients in bleaching materials are hydrogen peroxide and carbamide peroxide. These active substances may be used in different concentrations, depending on the type of bleaching

agent. Home bleaching materials are applied for extended periods and have low active substance content, while office bleaching materials are used for shorter periods of time and contain high active substance content. A number of studies have shown that bleaching materials affect the surface properties of composite resins during application for tooth whitening. This effect varies, depending on the matrix structure, the polymerization dynamics, the monomer properties of the composite resin, and the type of bleaching agent.⁹

Previous studies have investigated the effect of immersion in frequently consumed beverages on the surface properties and color of composite resins.¹⁰ However, there is not enough research on changes that can occur on the surface of the composite resin following bleaching after such immersion. The purpose of this study was to investigate the effect of staining and bleaching on surface microhardness and roughness, and color changes (ΔE) in microhybrid and nanohybrid composites. Our hypothesis was that staining and bleaching do not significantly affect the surface microhardness, roughness and color of composite resins.

Material and methods

Specimen preparation

The 2 types of composite resin and 3 different bleaching materials used in this study are shown in Table 1. An A2 color tone microhybrid composite (Gradia[®] Direct Anterior; GC Corporation, Tokyo, Japan) and a nanohybrid composite (Grandio[®]; VOCO GmbH, Cuxhaven, Germany) were selected for the research. One hundred and fifty specimens were obtained from each composite resin, using a Teflon mold, 2-millimeter-thick and 8 mm in diameter. The composites were placed inside the mold; then, a celluloid microscope slide holder strip was placed on the composite surface to obtain a smooth surface, and finger pressure was applied. The composite materials were then polymerized on their upper and lower surfaces for 20 s, using a light-curing unit (Elipar[™] FreeLight 2; 3M ESPE, St. Paul, USA) with a power of 1,000 mW/cm². The light intensity of the curing unit was checked using a digital radiometer (Hilux Ultra Plus; Benlioğlu Dental Inc., Ankara, Turkey) and the calibration of the light-curing unit was repeated for each group. After polymerization, the surfaces of the specimens were polished for 30 s, using a slow-speed handpiece with polishing discs (Sof-Lex[™]; 3M ESPE) under water.

Surface microhardness measurement

The surface microhardness of the composite resin specimens was tested after 24 h of storage in distilled water. A Vickers diamond indenter was used in a microhardness tester (Micromet[®] 2001; Buehler, Lake Bluff, USA)

Table 1. Resin composites and bleaching agents used in the study

Product	Type and contents	Manufacturer	Batch No.
Gradia Direct Anterior	microhybrid composite; Bis-GMA, UDMA filler vol. 66%	GC Corporation, Tokyo, Japan	1002121
Grandio	nanohybrid composite; Bis-GMA, TEGDMA, UDMA filler vol. 71.4%	VOCO GmbH Cuxhaven, Germany	1115259
Opalescence Boost	40% hydrogen peroxide	Ultradent Products Inc., South Jordan, USA	B63W8
Opalescence PF	35% carbamide peroxide	Ultradent Products Inc., South Jordan, USA	B3JW8
VivaStyle Paint On Plus	6% hydrogen peroxide	Ivoclar Vivadent AG, Schaan, Liechtenstein	M59447

Bis-GMA – bisphenol A-diglycidyl methacrylate; TEGDMA – triethyleneglycol dimethacrylate; UDMA – urethane dimethacrylate; vol. – volume.

for specimen indentation. Two random indentations were made on the top and bottom surfaces of each specimen, using a load of 10 g for 15 s for each microhardness test. All hardness values (HV) were calculated, where $1 \text{ HV} = 1.854 \text{ P/d}^2$, with P representing the indentation load and d the diagonal length.

Surface roughness measurement

The surface roughness (arithmetic mean roughness – Ra) of the specimens was examined with a contact mode profilometer. These values were obtained using the diamond tip of the profilometer (Surtronic® 25; Taylor Hobson Ltd, Leicester, UK), 5 µm in radius, with a cut-off value of 0.25 mm, a transverse length of 1.25 mm, a range of 100 µm, and a speed of 1 mm/s. This procedure was performed on 3 different sites, and the mean Ra values were obtained for each specimen.

Color change measurement

Specimen color measurements were carried out using a spectrophotometer (ShadePilot™, DeguDent GmbH, Hanau-Wolfgang, Germany) and were recorded as CIE L* a* b* values. The device was calibrated with its own calibration scale before starting color measurements in each group. Measurements were performed against a standard white background (L = 91.2; a = -0.6; and b = 1.4), and the mean CIE L* a* b* values from 3 measurements were obtained for each specimen. Color differences in the composite specimens were calculated using the following formula (Equation 1):

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad (1)$$

where:

L_1^* , a_1^* , b_1^* – initial CIE L* a* b* values of the composite specimens; and

L_2^* , a_2^* , b_2^* – CIE L* a* b* values measured at time periods ($\Delta L = L_2^* - L_1^*$; $\Delta a = a_2^* - a_1^*$; and $\Delta b = b_2^* - b_1^*$).

Staining procedures

After the initial color, microhardness and roughness measurements had been taken, the specimens of each composite were divided into 5 groups, with 30 specimens in each group. The specimens were immersed for 30 days in tea (Yellow Label Tea; Lipton, Istanbul Turkey – a pre-fabricated tea bag was immersed in 150 mL of boiling water for 5 min, pH 5.78), coffee (Nescafé Classic 3in1; Nescafé, Bursa, Turkey – 3 g of coffee powder was dissolved in 150 mL of boiling water, pH 6.12), cola (The Coca-Cola Company, Istanbul, Turkey – pH 2.51), red wine (DLC Öküzgözü 2009; Doluca, Istanbul, Turkey – pH 3.28), or in distilled water (pH 6.47) as a control group. The specimens were stored in the staining solutions for 3 h per day and in distilled water for the rest of the day, at room temperature. The solutions were changed daily. The color measurements of the specimens stored in the staining solutions were repeated at 24 h, and at 7, 15 and 30 days. After 30 days of storage, microhardness and roughness measurements were performed on the surface of each specimen.

Bleaching procedures

After the completion of the staining period, the specimens stored in each solution were separated into 3 subgroups ($n = 10$). One of these subgroups was bleached with the 40% Opalescence™ Boost (OB) office bleaching agent (Ultradent Products Inc., South Jordan, USA) (20 min twice per week for 14 days), the second one with the Opalescence PF (OP) home bleaching agent (Ultradent Products Inc.) (30 min per day for 14 days) and the third with VivaStyle® Paint On Plus (VP) home bleaching agent (Ivoclar Vivadent AG, Schaan, Liechtenstein) (10 min twice per day for 14 days). After the 2-week bleaching period, color, microhardness and roughness measurements were repeated on the surfaces of the composite resin specimens. The value $\Delta E = 3.3$ was determined as the clinically acceptable color change limit.

Statistical analyses

The data obtained from color, microhardness and roughness measurements was recorded and subjected to statistical analyses, using the PASW Statistics for Windows, v. 18.0, software (SPSS Inc., Chicago, USA). The surface microhardness and roughness data was compared using the repeated-measures analysis of variance (ANOVA) with 2 materials, 5 staining solutions and 3 bleaching agents, and by examining all interactions between them. Significant results were evaluated using the Bonferroni test ($p < 0.05$).

Results

The ANOVA results for microhardness measurements are shown in Table 2 and Table 3. Statistically significant differences were found in surface microhardness between different composite specimens subjected to staining and bleaching

Table 2. Analysis of variance (ANOVA) results for surface microhardness measurements

Source	Type III sum of squares	df	Mean square	F	p-value
Composites	467,531,607	1	467,531,607	15,726,326	0.000**
Solutions	551,787	4	137,947	4,640	0.002**
Bleaching agents	108,638	2	54,319	1,827	0.165
Composites * Solutions	746,020	4	186,505	6,273	0.000**
Composites * Bleaching agents	72,581	2	36,290	1,221	0.299
Solutions * Bleaching agents	576,774	8	72,097	2,425	0.018**
Composites * Solutions * Bleaching agents	779,029	8	97,379	3,276	0.002**
Error	3,567,508	120	29,729	–	–

df – degrees of freedom; ** statistically significant.

($p < 0.05$). The surface microhardness of the microhybrid composite specimens decreased significantly after bleaching ($p < 0.05$). The mean surface microhardness of the nano-hybrid composite specimens was significantly higher than that of the microhybrid composite specimens ($p < 0.05$).

The surface roughness measurement results are shown in Table 4 and Table 5. No statistically significant differences were found in surface roughness between the same composite specimens stored in different solutions and exposed to different bleaching agents ($p > 0.05$). In general, the mean Ra values of the nano-hybrid composite specimens were significantly higher than those of the microhybrid composite specimens ($p < 0.05$).

Color changes in the composite resin specimens after staining with different solutions and bleaching with different agents are shown in Table 6 and Table 7. The mean ΔE values for the microhybrid composite specimens were higher than those for the nano-hybrid composite specimens. The highest ΔE values in both composite groups

Table 4. Analysis of variance (ANOVA) results for surface roughness measurements

Source	Type III sum of squares	df	Mean square	F	p-value
Composites	8,842	1	8,842	129,301	0.000**
Solutions	0,029	4	0,007	0,108	0.980
Bleaching agents	0,333	2	0,166	2,431	0.092
Composites * Solutions	0,110	4	0,027	0,402	0.807
Composites * Bleaching agents	0,241	2	0,121	1,765	0.176
Solutions * Bleaching agents	0,468	8	0,059	0,856	0.556
Composites * Solutions * Bleaching agents	0,718	8	0,090	1,312	0.244
Error	8,206	120	0,068	–	–

** statistically significant.

Table 3. Mean surface microhardness values (HV) [P/d²] for the composite resins after staining and bleaching

Composite material	Measurement	Distilled water			Tea			Coffee			Cola			Red wine		
		OB	OP	VP	OB	OP	VP	OB	OP	VP	OB	OP	VP	OB	OP	VP
Gradia Direct Anterior	at baseline	43.4 ±2.73 ^a	45.1 ±5.80 ^a	50.2 ±3.05 ^a	49.6 ±4.05 ^a	45.8 ±3.64 ^a	46.4 ±3.19 ^a	46.4 ±4.85 ^a	46.0 ±5.31 ^a	47.8 ±2.45 ^a	48.0 ±3.80 ^a	45.7 ±3.79 ^a	47.9 ±1.75 ^a	50.6 ±2.60 ^a	46.6 ±3.22 ^a	49.0 ±2.65 ^a
	after staining	47.2 ±0.60 ^a	47.3 ±0.75 ^a	47.1 ±0.77 ^a	48.5 ±0.38 ^a	48.0 ±1.40 ^a	47.7 ±1.59 ^a	38.4 ±4.10 ^a	52.3 ±4.60 ^a	47.6 ±0.95 ^a	46.6 ±0.20 ^a	44.4 ±5.42 ^a	44.6 ±4.51 ^a	47.2 ±0.27 ^a	47.0 ±0.59 ^a	47.0 ±0.38 ^a
	after bleaching	30.5 ±1.18 ^b	29.8 ±1.03 ^b	31.4 ±2.51 ^b	32.8 ±0.64 ^b	32.2 ±0.96 ^b	55.7 ±7.88 ^a	36.1 ±7.22 ^a	50.6 ±7.27 ^a	32.2 ±1.48 ^b	32.5 ±1.31 ^b	31.3 ±0.68 ^b	31.3 ±2.23 ^b	28.9 ±0.58 ^b	32.2 ±1.13 ^b	32.0 ±2.22 ^b
Grandio	at baseline	110.9 ±4.62 ^a	113.3 ±4.69 ^a	112.6 ±4.57 ^a	104.0 ±4.74 ^a	112.5 ±5.52 ^a	110.7 ±3.30 ^a	110.3 ±7.80 ^a	112.8 ±6.93 ^a	114.4 ±2.12 ^a	110.3 ±2.61 ^a	108.8 ±4.52 ^a	105.9 ±4.26 ^a	109.4 ±3.51 ^a	111.6 ±2.33 ^a	108.6 ±6.02 ^a
	after staining	110.4 ±2.96 ^a	110.3 ±2.50 ^a	108.2 ±5.57 ^{ab}	107.9 ±6.93 ^a	102.1 ±5.78 ^a	103.4 ±3.96 ^b	112.9 ±4.74 ^a	107.7 ±4.73 ^a	110.5 ±6.50 ^a	105.4 ±3.63 ^{ab}	104.7 ±3.63 ^a	105.6 ±3.91 ^a	106.8 ±1.18 ^a	105.0 ±3.30 ^a	104.3 ±2.92 ^a
	after bleaching	110.8 ±3.32 ^a	109.4 ±10.90 ^a	104.7 ±2.57 ^b	100.5 ±5.75 ^a	103.7 ±6.13 ^a	107.8 ±2.81 ^{ab}	104.8 ±4.46 ^a	103.4 ±8.68 ^a	104.5 ±10.2 ^a	98.9 ±2.09 ^b	106.8 ±2.09 ^a	104.7 ±9.98 ^a	102.5 ±5.92 ^a	103.5 ±2.64 ^a	102.6 ±19.7 ^a

OB – Opalescence Boost; OP – Opalescence PF; VP – VivaStyle Paint on Plus; letters in superscript ^{a, b, ab} indicate differences between measurements for the same composite material and bleaching agent. Data presented as mean ± standard deviation ($M \pm SD$).

Table 5. Mean surface roughness (Ra) values [μm] for the composite resins after staining and bleaching

Composite material	Measurement	Distilled water			Tea			Coffee			Cola			Red wine		
		OB	OP	VP	OB	OP	VP	OB	OP	VP	OB	OP	VP	OB	OP	VP
Gradia Direct Anterior	at baseline	0.10 ± 0.02	0.09 ± 0.02	0.17 ± 0.04	0.10 ± 0.04	0.18 ± 0.04	0.14 ± 0.06	0.10 ± 0.01	0.11 ± 0.01	0.20 ± 0.07	0.10 ± 0.03	0.25 ± 0.09	0.11 ± 0.02	0.12 ± 0.03	0.14 ± 0.04	0.09 ± 0.02
	after staining	0.11 ± 0.03	0.09 ± 0.02	0.18 ± 0.04	0.10 ± 0.03	0.16 ± 0.06	0.20 ± 0.06	0.12 ± 0.00	0.12 ± 0.03	0.28 ± 0.09	0.11 ± 0.05	0.34 ± 0.09	0.17 ± 0.08	0.21 ± 0.25	0.19 ± 0.07	0.14 ± 0.04
	after bleaching	0.13 ± 0.09	0.17 ± 0.11	0.13 ± 0.05	0.21 ± 0.21	0.14 ± 0.05	0.26 ± 0.21	0.19 ± 0.14	0.14 ± 0.08	0.21 ± 0.15	0.16 ± 0.06	0.26 ± 0.19	0.22 ± 0.07	0.34 ± 0.24	0.24 ± 0.25	0.11 ± 0.06
Grandio	at baseline	0.42 ± 0.19	0.26 ± 0.13	0.43 ± 0.31	0.36 ± 0.25	0.24 ± 0.18	0.39 ± 0.24	0.49 ± 0.26	0.43 ± 0.16	0.38 ± 0.07	0.23 ± 0.16	0.24 ± 0.14	0.31 ± 0.12	0.23 ± 0.26	0.48 ± 0.14	0.29 ± 0.03
	after staining	0.45 ± 0.22	0.24 ± 0.14	0.40 ± 0.44	0.40 ± 0.71	0.28 ± 0.20	0.45 ± 0.56	0.47 ± 0.42	0.49 ± 0.29	0.40 ± 0.20	0.35 ± 0.19	0.20 ± 0.43	0.64 ± 0.13	0.22 ± 0.31	0.72 ± 0.43	0.42 ± 0.18
	after bleaching	0.52 ± 0.12	0.54 ± 0.21	0.74 ± 0.20	0.38 ± 0.27	0.65 ± 0.25	0.81 ± 0.23	0.34 ± 0.39	0.45 ± 0.26	0.68 ± 0.47	0.57 ± 0.58	0.54 ± 0.46	0.73 ± 0.25	0.46 ± 0.44	0.45 ± 0.46	0.61 ± 0.20

Data presented as mean \pm standard deviation ($M \pm SD$).

Table 6. Mean color change (ΔE) values of the composite specimens at different staining stages

Composite material	Solution	24 h	7 days	15 days	30 days
Gradia Direct Anterior	distilled water	1.7 \pm 0.9	1.6 \pm 0.9	1.5 \pm 0.6	1.8 \pm 1.0
	tea	2.1 \pm 0.9	2.2 \pm 0.8	3.5 \pm 0.7	4.4 \pm 0.4
	coffee	1.8 \pm 0.9	2.0 \pm 0.8	2.2 \pm 0.6	2.7 \pm 0.7
	cola	1.5 \pm 0.7	2.1 \pm 0.5	2.0 \pm 0.7	2.6 \pm 0.5
	red wine	3.2 \pm 1.0	5.9 \pm 0.9	7.2 \pm 1.1	9.4 \pm 1.2
Grandio	distilled water	1.8 \pm 0.8	1.4 \pm 0.8	2.1 \pm 0.8	2.0 \pm 1.1
	tea	2.7 \pm 1.0	3.5 \pm 1.1	4.3 \pm 1.0	5.7 \pm 1.1
	coffee	2.2 \pm 0.7	2.8 \pm 0.7	3.5 \pm 0.7	4.6 \pm 0.8
	cola	1.4 \pm 0.5	2.2 \pm 0.6	2.7 \pm 0.5	3.3 \pm 0.7
	red wine	3.6 \pm 1.3	6.4 \pm 1.0	8.3 \pm 1.2	10.4 \pm 1.0

Data presented as mean \pm standard deviation ($M \pm SD$).

were observed in the specimens stored in red wine. The mean ΔE values were higher than the clinically acceptable ones in the tea and red wine groups among the microhybrid composite specimens, and in all of the nanohybrid composite specimen groups, except for the control group. In general, the mean ΔE values decreased with bleaching applications and the highest decrease was found in the VP subgroups.

Discussion

In this study, the effects of staining and bleaching on the surface microhardness, roughness and color of microhybrid and nanohybrid composite resins were investigated. Considering the results, our hypothesis that staining and bleaching do not significantly affect the surface microhardness, roughness and color of composite resins was rejected. Restorative materials used in dentistry aim to mimic natural tooth structures. It is desirable for restorative materials to exhibit similar properties to dental tissues and contact enamel surfaces. It is also desirable that food, beverage, pH, and similar effects in the oral environment should not affect the surface properties of the restoration.¹¹

The microhardness test is an effective method of measuring the mechanical strength and rigidity of a material. Microhardness testing relies on the principle that a suitably selected static diamond tip will leave a mark on the tested material when it is applied to the material under a given load within a certain period.¹² The microhardness value is obtained by measuring the microscopic trace which occurs after the load has been removed. Different methods are used to measure surface hardness. Which method should be selected depends on the material to be tested. Since the Vickers test is suitable for measuring the hardness of brittle materials, it can be used to measure the microhardness

Table 7. Mean color change (ΔE) values of the composite specimens after bleaching

Composite material	Bleaching	Distilled water		Tea		Coffee		Cola		Red wine	
		before	after	before	after	before	after	before	after	before	after
Gradia Direct Anterior	OB	1.3 \pm 0.41	1.3 \pm 0.60	4.1 \pm 0.28	2.3 \pm 0.20	2.5 \pm 1.02	1.7 \pm 0.90	3.0 \pm 0.36	1.9 \pm 0.81	9.5 \pm 1.39	3.6 \pm 0.32
	OP	2.1 \pm 1.18	1.4 \pm 0.82	4.6 \pm 0.40	2.2 \pm 0.66	2.8 \pm 0.70	1.7 \pm 0.66	2.2 \pm 0.43	1.5 \pm 0.33	9.8 \pm 1.65	4.8 \pm 0.80
	VP	1.9 \pm 1.06	0.8 \pm 0.71	4.5 \pm 0.50	0.8 \pm 0.75	2.9 \pm 0.58	0.9 \pm 0.58	2.6 \pm 0.44	1.2 \pm 0.40	9.0 \pm 0.86	1.7 \pm 0.45
Grandio	OB	2.6 \pm 0.94	3.0 \pm 0.76	5.4 \pm 0.42	2.0 \pm 0.80	5.0 \pm 1.06	3.0 \pm 0.84	3.5 \pm 0.81	2.0 \pm 0.85	10.0 \pm 1.26	3.9 \pm 0.80
	OP	1.9 \pm 1.38	1.3 \pm 0.94	6.5 \pm 1.57	2.1 \pm 0.97	4.1 \pm 0.63	2.4 \pm 0.42	2.9 \pm 0.48	2.0 \pm 0.58	10.7 \pm 0.38	4.1 \pm 0.73
	VP	1.6 \pm 1.14	1.4 \pm 0.66	5.1 \pm 0.86	1.4 \pm 0.50	4.6 \pm 0.52	2.4 \pm 0.32	3.4 \pm 0.70	2.2 \pm 0.54	10.5 \pm 1.32	1.9 \pm 0.38

Data presented as mean \pm standard deviation ($M \pm SD$).

of composite specimens.¹³ Vickers hardness tester tips are shorter than those of other testers, which means that the testing device is less affected by the surface properties of the material, thus producing more accurate measurements.¹⁴ Due to these advantages, in this study, microhardness measurements were taken using a Vickers hardness tester.

The chemical composition and filler content of composite resins affect their physical properties, such as surface microhardness. In their examination of the mechanical properties of composite resins, Braem et al. reported that materials with high filler content had higher surface hardness.¹⁵ Similarly, Rodríguez et al. found that the mechanical properties of composite resins were related to the amount of filler employed.¹⁶ The mean HV of the nanohybrid composite resin specimens in the present study were significantly higher than those of the microhybrid composite resin specimens ($p < 0.05$). The filler content of the nanohybrid composite resin used in this study (71.4% by volume) is higher than that of the microhybrid composite resin (66% by volume). Surface hardness is also affected by the degree of polymerization. There are some studies in the literature that report that nanohybrid composites show lower degrees of polymerization than microhybrid composites.¹⁷ In the present study, the higher surface microhardness of the nanohybrid composite specimens may also be related to the degree of polymerization.

Statistically significant differences were observed in the surface microhardness of the composite resin specimens after staining ($p < 0.05$). The mean surface microhardness of the specimens stored in cola and red wine generally decreased. Low-pH beverages, such as cola, are reported to cause erosion and dissolution on the surface of composite resin specimens, and to reduce surface hardness. In addition, the surfaces of Bis-GMA- and UDMA-based polymers tend to undergo chemical softening under the effect of alcohol.¹⁸ A decrease in the mean surface microhardness of the specimens stored in red wine in this study can be explained in terms of this effect. Similarly, Okte et al. reported decreases in the mean surface microhardness of the specimens stored in red wine in their study.¹⁹

Bleaching agents may affect the surface microhardness of composite resins, depending on factors that include the composition of the material, the concentration of the bleaching agent and the method of application. Cehreli et al. found that the surface microhardness of composite resin specimens decreased with bleaching applications.²⁰ Okte et al. showed that bleaching applications reduced surface microhardness in microhybrid composites and that this was due to the oxidation effect of hydrogen peroxide in the bleaching agent on the resin matrix.¹⁹ The surface microhardness of the microhybrid composite specimens in the present study decreased significantly as a result of bleaching applications ($p < 0.05$). The mean surface microhardness of the nanohybrid composite specimens decreased; however, the differences

were not statistically significant ($p > 0.05$). The bleaching agents used in this study, containing hydrogen peroxide and carbamide peroxide at different concentrations, reduced the mean microhardness of the composite resin specimens by affecting the surface matrix structure.

As the surface roughness of the restoration decreases, its appearance improves, coloring resistance and abrasion resistance increase, plaque deposition decreases, and the health of the periodontal tissues is maintained. In addition, as the surface smoothness of the restoration increases, microleakage between the tooth and the restoration decreases, and the risk of secondary caries declines. Studies have also proven that oral hygiene increases in line with the surface smoothness of the restoration.²¹ The most commonly used parameter for measuring the surface roughness of composite resins is Ra. Profilometer devices have long been used to obtain this value from the material surface in vitro. These devices are capable of calculating the mean Ra values of various materials by capturing two-dimensional (2D) images from the specimen surface.²²

In the literature, some studies have indicated that the surface properties of the composite resin develop as the filler particle size decreases. These studies suggest that nanoscale particles form a smoother surface as compared to the conventional microhybrid composites.^{23–25} Ergücu et al. reported that smaller defects occurred on the surfaces of composite resins after polymerization and polishing, and that smoother surfaces were obtained.²² Similarly, Ereifej et al. found that nanofiller composites provided lower surface roughness and better polishability.²⁶ In contrast to these studies, the mean Ra values of the nanohybrid composite specimens in the present study were higher than those of the microhybrid composite resin specimens. Although the primary particle size of the nanohybrid composite used in this study is very small, these particles aggregate in larger masses. This structure may increase the surface roughness of the nanohybrid composite. Other important factors affecting the surface roughness of composite resins in addition to the filler particle size include the type and amount of filler, and the organic matrix structure.²⁶

The exposure of composite resin restorations to different solutions in the oral environment may also affect their surface properties. In the present study, the composite resin specimens were stored in commonly used alcoholic (red wine) and non-alcoholic (tea, coffee and cola) solutions, and their Ra values were compared with those of the control group. Although no statistically significant change in Ra values was observed after staining in either composite, the mean surface roughness increased, especially in the specimens stored in cola and red wine. Previous studies have shown that alcoholic beverages increase the surface roughness of composite resins.²⁷ Alcohol derivatives, such as ethanol in red wine, can increase the surface roughness of the composite resin by penetrating the resin matrix structure. Bansal et al. investigated

the effect of different solutions on the surface roughness of composite resins and observed the highest Ra values in the specimens stored in cola.²⁷ They attributed this to the low pH of cola, which leads to wear and roughness on the surface.²⁷ In the present study, an increase in the mean surface roughness of the specimens stored in alcohol and cola may be explained by the fact that these drinks cause a slight degradation of the resin matrix structure.

The surface roughness of the composite resins in the present study increased with the application of different bleaching agents, although this increase was not statistically significant ($p > 0.05$). Similar to our study, Türker and Biskin also reported that carbamide peroxide-containing agents increased the surface roughness of composites, but that this increase was not statistically significant.²⁸ Bowles et al. reported that bleaching agents containing hydrogen peroxide did not affect composite surface roughness.²⁹ Consistent with our results, that study concluded that bleaching agents slightly impaired the composite structure through oxidation, but that this was not statistically significant.²⁹

Color plays an important role in the success of esthetic restorations. In order to provide a pleasing cosmetic appearance for an extended period of time, the color of the restorative material must be compatible with the tooth color and resist coloring.³⁰ Discoloration can be assessed using different methods, such as visual or instrumental techniques. Seghi et al. reported that color change could only be perceived with difficulty at a ΔE value equal to or lower than 1, whereas a ΔE value greater than 2 could usually be detected clinically.³¹ Johnston and Kao investigated color differences with the use of visual evaluation and colorimetry, and they reported $\Delta E = 3.3$ as the threshold value at which color differences can be distinguished in the oral environment.³² The generally acceptable clinical ΔE value in the literature is 3.3, since a lower ΔE value will be visually imperceptible.³³ A spectrophotometer equipped with an integrating sphere can be used to measure ΔE in composite resins. This configuration of a spectrophotometer yields a more accurate result than visual assessment or other instruments.³⁴ Due to these advantages, a spectrophotometer was employed for color measurement in this study.

The mean ΔE values of the nanohybrid composite specimens before and after staining were higher than those of the microhybrid composite specimens. Water absorption can lead to color change in restorations. In addition, absorbing excess water, composite resins can expand and plasticize, which results in the formation of microfractures. Microfractures or voids in the interface between the filler and the matrix thus cause stain penetration and color change. The chemical properties of composite resins therefore directly affect color change. The hydrophilic TEGDMA molecule has been reported to increase the water absorption of composite resins, thus causing discoloration. In addition to water absorption,

the surface roughness of composite resins is an effective factor in stain retention.³⁵ In the present study, the monomer content and the higher mean surface roughness of the nanohybrid composite resin specimens may explain the higher mean ΔE values observed.








The highest ΔE values in the present study were observed, in descending order, in the specimens stored in red wine, tea and coffee. Similar to our study, Stober et al.³⁶ and Ertaş et al.³⁷ reported that the highest color change was caused by red wine followed by tea and coffee. Um and Ruyter reported that the low pH of cola affected the surface integrity of the composite resin, thus causing less discoloration than tea and coffee.³⁸ The exposure time is also important in staining composite resins. In the present study, the clinically unacceptable color change was observed after 24 h in the specimens stored in red wine, and after 15 days in those stored in tea and coffee.

The mean ΔE values of the bleached specimens decreased below the clinically acceptable threshold in this study, except for those kept in red wine. The lowest ΔE values after bleaching were observed in the VP specimens. This bleaching agent contains fewer active ingredients than the others (6% hydrogen peroxide). Since OB and OP are gel-like agents, they were easily removed from the specimen surfaces by washing. However, since VP is a highly adhesive agent, it could not be completely removed from the specimen surfaces and may have exerted a more prolonged effect than the other agents over a 14-day period. This may explain the lower ΔE values of the VP subgroups. Bleaching agents penetrate from the surfaces of the teeth and exert an oxidizing effect. When they are applied to the surfaces of composite resins, they perform only superficial cleaning and do not affect the internal coloration. This situation is manifested by the stained composite returning to its original color.³⁹ In the present study, it was determined that color change that occurred in the specimens after staining decreased after bleaching.

Conclusions

According to the results of the present study, staining and bleaching processes may cause changes in the microhardness, roughness and color of composite resin surfaces, depending on structural properties, such as the type of composite material, its matrix structure and filler content. Although bleaching is effective in cleaning the composite surface, it cannot act in the same way as in the case of dental tissues, and bleaching agents may also affect the surface properties of composite resins. Therefore, bleaching agents should be applied carefully in order to remove staining on restored tooth surfaces. If the bleaching agent is found to degrade the surface properties of the old restoration, or if a color difference is observed between the tooth and the restoration, the old composite resin restoration should be replaced after bleaching.

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COVID-19 pathology imaging: A one-year perspective

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Abstract

The first cases of coronavirus disease 2019 (COVID-19) were reported in Wuhan, China, in December 2019. Five months later, the World Health Organization (WHO) announced a pandemic. The symptoms are non-specific, and include breathing difficulties, cough, fever, and the loss of smell and taste. The diagnosis is confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) testing. Medical imaging has been mainly used to estimate the range of disease or potential complications.

The aim of this study was to present the radiographic features of COVID-19 reported in published papers. This investigation includes the scientific work concerning chest radiography (chest X-ray – CXR) and computed tomography (CT) in COVID-19 patients. The most common pathologies are described, and the classification of COVID-19 appearance in CT and other radiology reports is summarized. The usage of lung ultrasound (LUS) was taken into consideration. This study emphasizes the role of artificial intelligence (AI) in the COVID-19 pandemic. The algorithms developed to detect the disease are discussed. The role of medical imaging is not limited to the respiratory system; it can also be used in searching for and monitoring complications (cardiac, vascular or brain damage). Due to the significant role of radiology in the current pandemic, a review of the latest medical literature was performed to help clarify the upcoming data.

Keywords: ultrasound, computed tomography, radiography, COVID-19, artificial intelligence

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Introduction

Coronavirus disease 2019 (COVID-19) is a medical condition caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In March 2020, the World Health Organization (WHO) declared the disease a pandemic.¹ Globally, as of December 2020, the number of COVID-19 cases was over 75 million and so far the disease has affected almost every territory. Worldwide, the current number of deaths exceeds 1.6 million.² The symptoms and signs of the infection are non-specific, and may include fever, cough, anosmia, and fatigue. Coronavirus disease 2019 mainly affects the respiratory system; therefore, the role of thoracic radiology should be established. The COVID-19 diagnosis is based on the real-time reverse transcriptase-polymerase chain reaction (RT-PCR) test.³ Due to the symptoms of pneumonia, some patients require medical imaging. This study aimed to systematize the radiographic features presented in a traditional radiograph, computed tomography (CT) and lung ultrasound (LUS). Based on medical imaging data, many artificial intelligence (AI) algorithms have been developed to help in the detection of COVID-19. During the last year, multiple articles highlighting the radiological findings in COVID-19 patients were published. Many of these studies focused on a particular method of imaging or a specific type of disease complication. The role of this study was to systematize these results and conclusions for optimal use in an everyday clinical setting.

Methodology

The goal of this article was to provide an overview of COVID-19 pathology imaging. A review of all the relevant literature published in 2020 as well as of the current statements of the European Society of Radiology (ESR), the Radiological Society of North America (RSNA) and the British Society of Thoracic Imaging (BSTI) were performed. The sources of the utilized data were PubMed and Google Scholar databases. Only articles in English were included.

Chest radiography

Chest radiography (chest X-ray – CXR) is less sensitive than chest CT. However, due to the limited availability and high costs of CT, CXR is performed first. Furthermore, in some countries, CXR cannot be followed up by CT, especially during a pandemic. For this reason, it is essential to describe the radiographic features which can be seen on a chest radiograph, not only by a radiologist, but also other specialists.⁴ Chest radiography in early and mild cases of COVID-19 may



Fig. 1. Chest radiography (CXR). Bilateral pneumonia in a patient with confirmed coronavirus disease 2019 (COVID-19)

not reveal any pathologies. Studies from China showed that only 54.2% of non-severe disease cases presented with abnormalities on chest radiographs; in severe cases, almost 77% of patients had an abnormal chest



Fig. 2. Chest computed tomography (CT). Ground-glass opacities (GGOs) (arrows) in a patient with confirmed COVID-19

radiograph.⁵ The most common radiographic features are patchy or diffuse asymmetric air-space opacities, consolidations, and ground-glass opacities (GGOs).⁶ The distribution of these features is frequently bilateral. The peripheral and lower zone distribution are the prevalent locations of these signs (Fig. 1). Pleural effusion is a rare complication experienced by approx. 3% of patients. In these cases, there is no pathognomonic appearance of COVID-19 in the chest radiograph and the image is similar to other pneumonias.⁴ Due to this fact, CXR is not recommended for screening or use as a diagnostic tool for COVID-19. It should be noted that portable imaging equipment limits the transportation of patients and minimizes the risk of spreading the disease.⁷ Reports from radiology departments, including the University of Washington and Australian hospitals, highlighted a technique whereby an X-ray can be performed through a glass window and the patient does not leave the isolation room.^{8,9}

Chest computed tomography

In the early stage of COVID-19, the primary CT findings observed are bilateral GGOs with a mostly peripheral and subpleural location. Ground-glass opacity is a term used to describe hazy areas with the

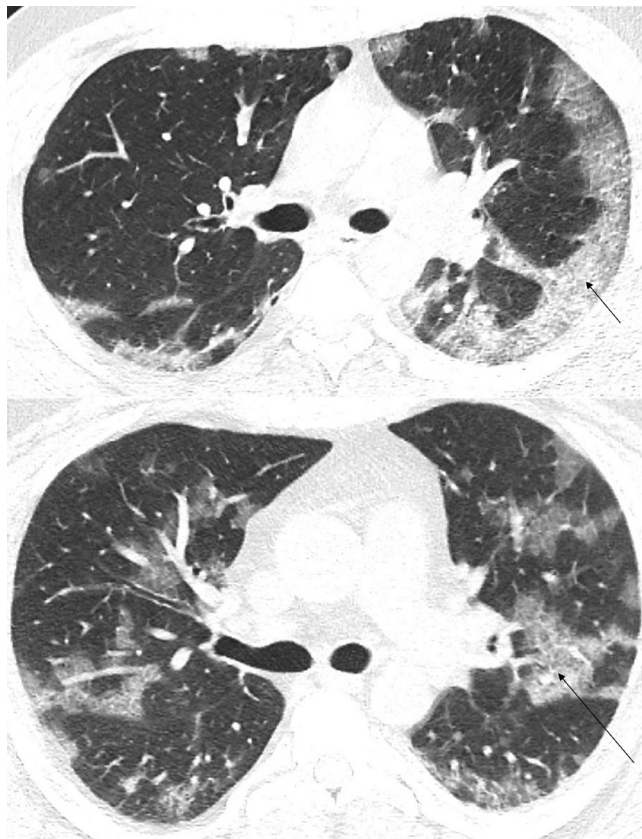


Fig. 3. Chest CT. Crazy-paving appearance (arrows) in a patient with confirmed COVID-19

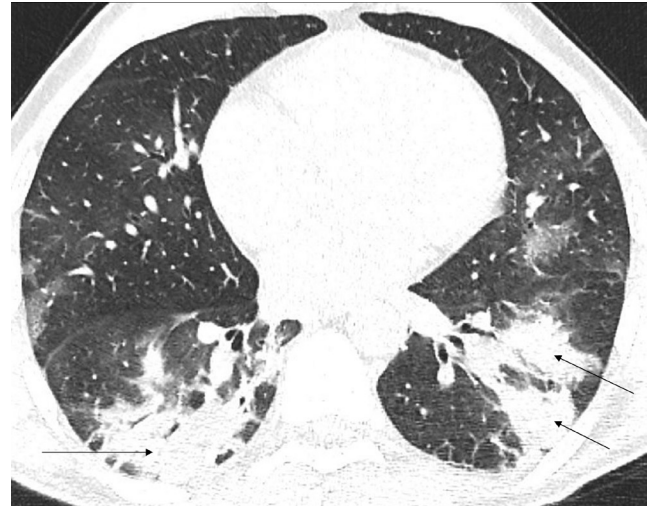


Fig. 4. Chest CT. Consolidations (arrows) in a patient with confirmed COVID-19

preservation of vascular and bronchial margins which are a result of the partial filling of air spaces, interstitial thickening, or the coexistence of both (Fig. 2).¹⁰ Another phenomenon is described as having ‘crazy-paving’ appearance. This is a combination of GGO and interlobular or intralobular septal thickening (Fig. 3). This condition is mostly present in severe cases of COVID-19 pneumonia. Consolidations (multifocal, patchy or segmental) are another sign of COVID-19 progression (Fig. 4).¹¹ Several days after developing the first symptoms, chest CT can reveal linear consolidations and reversed halo signs in the patient’s lungs.¹² Radiographic features typically include bronchovascular thickening (Fig. 5).¹³

Recently, COVID-19 progression has been described with successive CT scans and has been correlated with the onset of symptoms. Shi et al. monitored a patient for 3 weeks and performed 4 CT scans.¹⁴ The 1st CT scan,



Fig. 5. Chest CT. Bronchovascular thickening (arrow) in a patient with confirmed COVID-19

obtained 9 days after the onset of symptoms, revealed multifocal bilateral GGOs. After 15 days of disease evolution, a mixed pattern of GGOs and consolidations was observed. The signs of healing were seen after 19 days. The last CT scan was performed on day 31 and no abnormalities were found.¹⁴

A retrospective study of 121 symptomatic patients infected with SARS-CoV-2 was performed by Bernheim et al.¹⁵ The main aim of their research was to assess the correlation between the onset of symptoms and common findings on the initial CT scan. The time between the occurrence of the first symptoms and subsequent CT scans was divided into 3 periods: early (0–2 days); intermediate (3–5 days); and late (6–12 days). Bilateral lung involvement, which is considered typical in COVID-19 pneumonia, was observed in 28% of early-stage patients, 76% of intermediate-stage ones and 88% of late-stage patients. Crazy-paving appearance and the reversed halo effect were absent in the early group, but were mostly present in the late-stage group. It was found that 56% of the early patients showed the lack of lung capacities during the radiological examination despite the presence of clinical symptoms. The study results exhibit imaging patterns, essential not only for understanding the pathophysiology or natural history of COVID-19, but also for the prediction of its progression and potential complications.¹⁵

Based on similar research, 4 stages of CT imaging have been established. The 1st stage is named the early or initial stage and lasts up to 4 days. A CT scan performed during this period may not reveal any pathologies or may only indicate the presence of GGOs. It is crucial to remember that up to half of patients may have a normal CT scan 2 days after the symptoms occur. The next phase is the progressive stage (5–8 days) – several GGOs arise and the crazy-paving pattern becomes vis-



Fig. 6. Chest CT. Fibrous stripes (arrows) in a patient with confirmed COVID-19

ible. Between 9 and 13 days, consolidations appear. This phase is called the peak stage. After 14 days, absorption begins and fibrous stripes may occur (Fig. 6). After at least 1 month, and if no other complications arise, the patient's lungs are able to return to a condition similar to that before COVID-19.¹⁶

Tomographic reporting methods and CO-RADS

Due to the never-before-seen scale of pneumonia and the rising amount of CT scans, creating a standardized reporting language for radiologists has become necessary. The BSTI published a report with suggestions regarding plain radiography.¹⁷ In cooperation, the RSNA, the American College of Radiology (ACR), and the Society of Thoracic Radiology (STR) released a special statement.¹⁷ The statement aimed to present a classification of COVID-19 appearance derived from CT. This classification suggests 4 types of images: typical; indeterminate; atypical; or negative for pneumonia. The typical appearance includes GGOs, consolidations, the crazy-paving pattern, and the reverse halo effect. Pathologies are located peripherally and bilaterally. The indeterminate appearance is characterized by the absence of typical CT findings, but the presence of other pathologies, including multifocal, diffuse, peripheral, or unilateral GGOs with or without consolidations, and a few small GGOs with non-rounded and non-peripheral distribution. The atypical appearance does not consist of typical or indeterminate features, but includes yet other pathologies. In this category, the presence of isolated lobar or segmental consolidations, without GGOs, is acceptable. Lung cavitation, discrete small nodules and smoother interlobular septal thickening with pleural effusion may occur. Regarding the last category, these include patients with no signs of COVID-19 pneumonia.¹⁷ This chest CT classification is widely used for clinical practice and subsequent studies.¹⁸ The Dutch Association for Radiology introduced a dedicated COVID-19 scoring system.¹⁸ It was named CO-RADS as a short version for COVID-19 Reporting and Data System. The role of this classification is to estimate the suspicion of COVID-19 pneumonia. Number 1 is reserved for a lack of probability. Higher numbers (up to 5) refer to typical COVID-19 features. CO-RADS 0 describes an incomplete examination or insufficient quality of CT. CO-RADS 6 is associated with a confirmed positive RT-PCR test.

Nowadays, various radiological societies and organizations remind us that CT should not be included in diagnostic and screening tools for COVID-19. Computed tomography scans should be reserved for patients in a serious condition to better estimate the stage of the disease as well as its progression or potential complications, and if RT-PCR is currently unavailable.¹⁹

Chest ultrasound

Lung ultrasound has recently become the recommended examination for acute respiratory failure.²⁰ In COVID-19 patients, early investigations from China suggest the promising utility of LUS in a 12-zone method.²¹ Characteristic findings included various forms of B-lines (focal, multifocal and confluent). The LUS of many patients with COVID-19 pneumonia revealed an unusual artifact. It has been described as a shining band-form artifact arising from a regular pleural line.²¹ The first Chinese researchers compared this artifact to a waterfall. In further scientific works, the term “waterfall” was replaced with “light-beam sign”.²² This artifact correlates with GGOs, which can be seen on CT scans.²³ Lung ultrasound can also reveal a thickened and irregular pleural line. Ultrasonographic features include various types of consolidations – multifocal, both non-translobar and translobar with air bronchograms. Pleural effusion may be observed during the examination, but it is not a common pathology. The observed features are related to the stage of the disease and change along with its duration. In early or mild infections, focal B-lines are frequently present. The LUS of COVID-19 patients in a critical condition shows alveolar intestinal syndrome (AIS). A-lines appear during recovery and uneven B-lines can occur as a sign of pulmonary fibrosis. Lung ultrasound can have a crucial role in the diagnosis and monitoring of patients with COVID-19. The main advantages are safety and the lack of radiation. This means unlimited access, due to the lack of exposure to ionizing radiation on one hand, and economic reasons on the other hand, and repeatability. Moreover, examinations can also be performed daily.²⁴ In addition, the patient avoids potentially dangerous transport to the radiology department.

Complications

COVID-19 may result in a number of complications ranging from mild to very severe cases. It is estimated that 17–29% of patients develop acute respiratory distress syndrome (ARDS).⁶ Although the disease mostly affects the respiratory system, complications may influence any other system. Commonly reported sequelae are acute cardiac injuries. The mechanism of cardiovascular injury from COVID-19 has not been completely explained and could be a complex phenomenon. However, it manifests itself by myocardial ischemia and cardiac arrest. Recent works describe not only elevated troponin levels, but also myocardial ischemia and cardiac arrest associated with COVID-19 infection.²⁵ The authors of the editorial entitled “Coronavirus disease 2019 (COVID-19) and the heart – Is heart failure the next chapter?” point to various cardiac complications after pneumonia.²⁶ The diagnosis of cardiovascular disease (CVD) complications should be based on examination, symptoms, elevated cardiac markers, and electrocardiography (ECG). However, when the patient’s condition is not stable, further steps must be taken, including medical imaging²⁷ (e.g., a CT scan²⁸). A severe COVID-19 complication is myocarditis (Fig. 7). Puntmann et al. suggest that approx. 60% of patients could experience myocardial inflammation, with no association to preexisting conditions or the severity of COVID-19.²⁹ When myocarditis is suspected, the examination that should be taken into consideration is cardiac magnetic resonance (CMR).²⁹ It is confirmed that SARS-CoV-2 infection increases the risk of pulmonary embolism (Fig. 8) and deep vein thrombosis.³⁰ When acute thromboembolic disease is suspected, not only should D-dimer levels be tested, but an assessment via computed tomography angiography (CTA) is essential.³¹

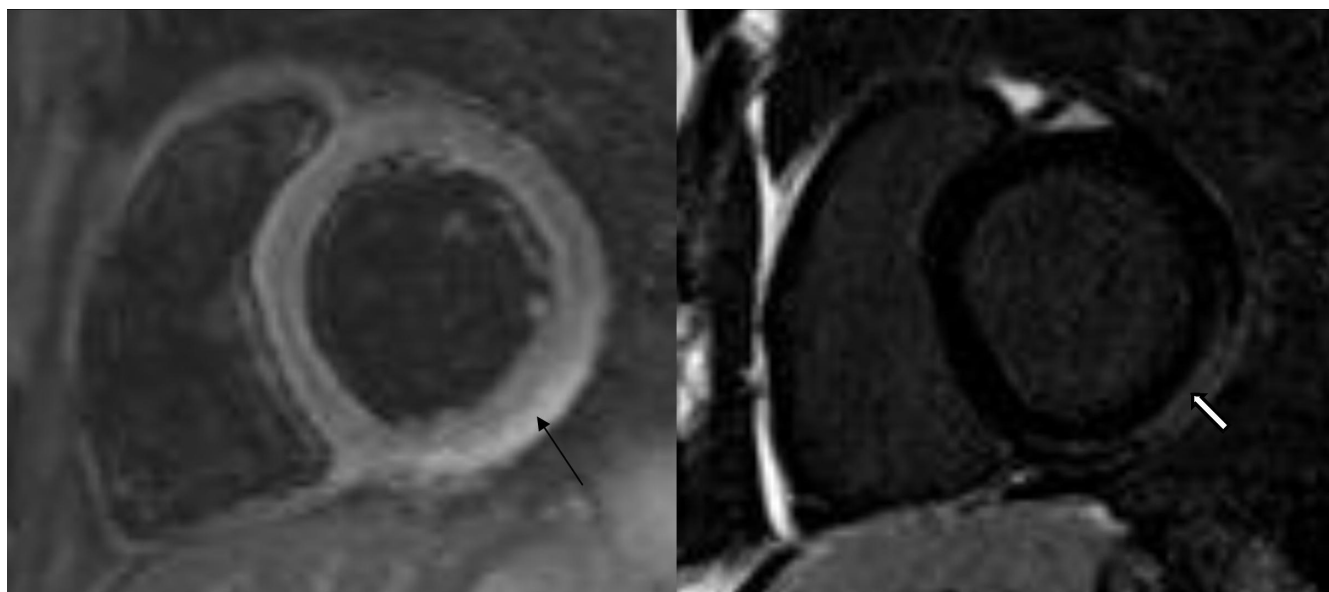


Fig. 7. Cardiac magnetic resonance (CMR). Myocardial edema in the STIR sequence (black arrow) and the area of late gadolinium enhancement in the LGE sequence (contour of black arrow) during active myocarditis

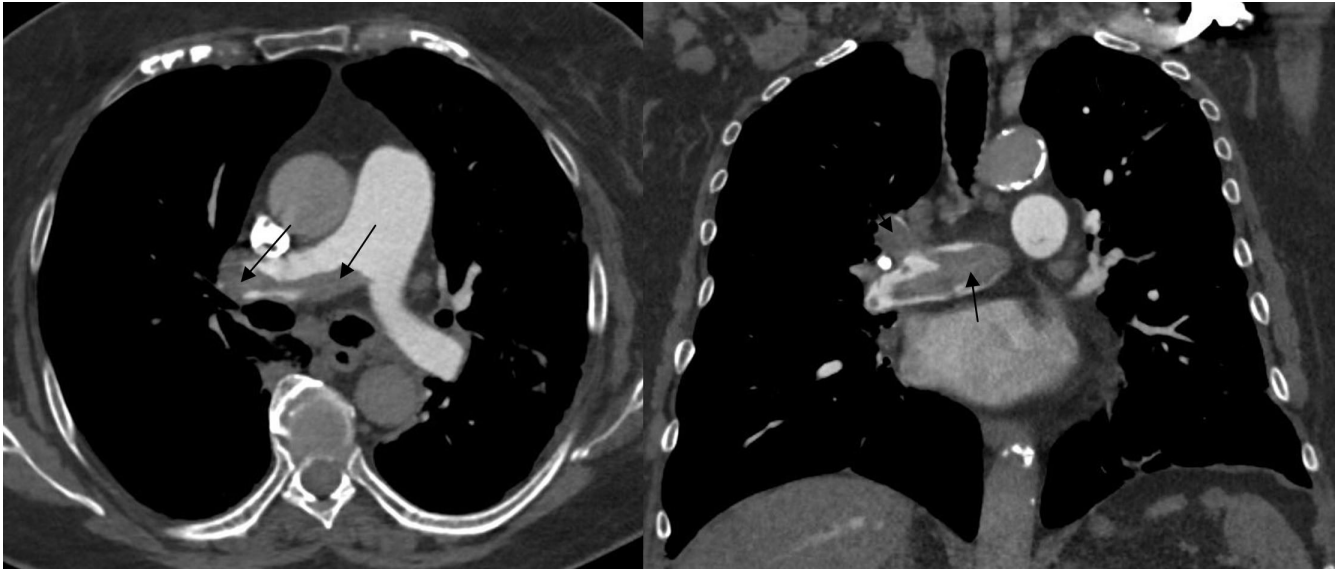


Fig. 8. Pulmonary computed tomography angiography (CTA). Loss of pulmonary artery filling (arrows) during acute pulmonary embolism

COVID-19 patients are at an increased risk of brain tissue damage. The injury of heart or brain tissue can be caused via 4 different pathways. These include the neuronal pathway, the hypoxia pathway, the renin–angiotensin–aldosterone system (RAAS) pathway, and the immune pathway. Patients with comorbidities are a high-risk group and need to be kept under careful observation. In case brain hemorrhage is suspected, CT or magnetic resonance imaging (MRI) should be performed (Fig. 9).³² Medical imaging is an essential tool in screening and localizing life-threatening COVID-19.



Fig. 9. Head CT. Intracranial hyperdense foci corresponding to hemorrhagic stroke (black arrow) and cerebral hematoma (contour of black arrow)

Artificial intelligence

The global pandemic has forced the medical industry to search for new technologies to estimate and control the spread of the new virus. During periods of peak infection, global healthcare infrastructure deteriorated rapidly. The main issue was staff shortage in many departments, including radiology. When the scale of the problem became unmeasurable, medical organizations decided to use a potentially vital, but still unexplored area of science, i.e., artificial intelligence (AI).³³ The global crisis allowed AI to be implemented in many facets of healthcare and epidemiology at the same time. These applications included tracking the spread of the virus or identifying high-risk patients.³⁴ However, due to the lack of radiologists, few programs using AI to diagnose COVID-19 were developed.

The main tasks for AI applications in radiology are the accurate detection of COVID-19 based on CXR or CT images, the differentiation of COVID-19 pneumonia from other kinds of pneumonia, and the assessment of the severity of the process in correlation with the patient's clinical status. Programs based on several pre-trained networks gained high diagnostic sensitivity and specificity, ranging from 84% to 100% and from 71% to 100%, respectively.³⁵ The high specificity for the differentiation of diagnosis between COVID-19 and non-COVID19 pneumonia via AI is promising.³⁶ In most cases, the algorithms were based on deep neural network learning, whilst some were based on machine learning with the use of thousands of radiologic images as a comparator. However, these data are heterogeneous due to the fact that they were obtained from different medical units, with the application of different equipment and slightly different protocols. Despite the encouraging results, the AI-based diagnosis of radiographic evidence should be considered as early data that

requires validation and further standardization before it can be used in clinical practice.³⁵

Although the role of AI in medicine is constantly developing, some of the benefits can already be highlighted. Doctors are limited with time; on the other hand, AI technologies can process an incomparable amount of data (i.e., X-ray scans) in a short period. In addition, the global machine learning research community enables sharing the data and innovations. As the pandemic develops and the disease spreads all over the world, access to up-to-date information is vital.³⁷

Artificial intelligence allows the processing of various types of information from the patient's medical history. It can correlate data like medical imaging, laboratory results, and clinical observations to conclude the most probable course of the disease and suggest further actions. When access to medical services is limited, systems based on telehealth may help. Patients can receive the help they require without leaving their homes, which stops the spread of the virus. Artificial intelligence may provide preliminary healthcare education and advice to COVID-19 patients. Additionally, it can inform on preventive measures.³⁸


Summary


Typical medical imaging in COVID-19 patients includes CXR, CT and LUS. Chest radiography should not be used for the diagnosis and screening of COVID-19. However, it may be performed in severe cases of the disease. The most common findings are patchy or diffuse asymmetric air spaces, consolidations, and GGOs. Similar pathologies can be described in chest CT. Previously, CT was used to monitor the condition of patients. In the first stage of the disease, any pathologies may not be revealed; however, after a few days (up to 8 days), GGOs and the crazy-paving pattern are the most common signs. Next, consolidations become visible. During the last period of COVID-19, fibrous stripes appear. Computed tomography scans are useful to estimate the range of disease, its progression and any complications. Lung ultrasound is a tool that is recommended for monitoring patients and has a growing role in diagnosis. Focal B-lines are present in early infection and A-lines are visualized during recovery; a thickened and irregular pleural line, various types of consolidations and an artifact called a light-beam sign may be also seen. The role of radiology is not only limited to the imaging of the lungs, but it can also be used in other organs. Computed tomography angiography is performed when acute thromboembolic disease occurs. Cardiovascular magnetic resonance is another useful tool to assess cardiac complications. When brain tissue damage is suspected, MRI is essential. Medical imaging is irreplaceable in the diagnosis of COVID-19-positive patients; however, it is essential to


perform it in a specific and directed situation. This study aimed to set in order the most common COVID-19 imaging methods, but also to match the appropriate imaging method to specific patient cases. It is crucial to perform an appropriate test when it is needed (e.g., in case of possible COVID-19 complications). It is also necessary to note that X-ray exposure should be avoided, as in COVID-19 screening, CXR is not recommended, but is still performed in many medical wards. Lastly, AI is emerging as a new healthcare solution that is capable of analyzing large amounts of data. Furthermore, AI can correlate this data with the imaging data from the clinic.

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Impact of dental plaque control on the survival of ventilated patients severely affected by COVID-19 infection: An overview

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Abstract

This overview was conducted to highlight the importance of adequate oral hygiene for patients severely affected by coronavirus disease 2019 (COVID-19) due to infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). These are patients who were admitted to the intensive care unit (ICU) to receive oxygen through mechanical ventilation due to severe pneumonia as a complication of COVID-19. Various dental plaque removal methods for ventilated patients were discussed with regard to their efficacy. The use of chemical agents was also considered to determine which one might be proposed as the best choice. Also, oral care programs or systems that can be implemented by ICU nurses or staff in the case of these ventilated patients were suggested based on evidence from the literature. These interventions aim to reduce microbial load in dental plaque/biofilm in the oropharynx as well as the aspiration of the contaminated saliva in order to prevent the transmission of the dental plaque bacteria to the lungs or other distant organs, and reduce the mortality rate.

Keywords: COVID-19, mechanical ventilation, ventilator-associated pneumonia, oral hygiene care, cytokine storm

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Introduction

The development of non-oral diseases may be promoted directly or indirectly by periodontal pathogens. Gram-negative anaerobic bacteria, which can be found in abundance in the oral cavity, release endotoxins that may directly contribute to the development of systemic diseases. This association was described by Socransky et al. as early as in 1998.¹ These oral pathogenic bacteria may reach other organs, such as the lungs, by direct inoculation or travel to distant organs via the bloodstream or the lymphatic system. Bacterial accumulation on the teeth due to inadequate oral hygiene, with or without other environmental factors, may induce not only the host inflammatory response, with the possible consequences of bone loss and subsequent periodontitis,² but also become systemically detrimental to the individual.

Coronavirus disease 2019 (COVID-19) was first discovered in Wuhan, China, at the end of 2019. Unfortunately, COVID-19 spread rapidly on a global scale and led to a full-blown pandemic that was announced by the World Health Organization (WHO) and the Public Health Emergency of International Concern (PHEIC) in March 2020. The first confirmed death associated with COVID-19 was in Wuhan on January 9, 2020³ and the first death outside of mainland China was reported in February in the Philippines.⁴ The first casualty outside of Asia occurred in France and was recorded on February 14, 2020.³ By the end of February, many more deaths related to COVID-19 had been recorded in countries outside of mainland China, including Iran, South Korea and Italy. By March 2020, when the disease was declared a pandemic, many more countries had reported deaths caused by COVID-19.⁵ As of September 30, 2020, according to WHO, the death toll due to COVID-19 had reached 1,004,421.⁶

Methodology

The relevant literature was selected from 3 databases, including Web of Science, PubMed and Google Scholar searches. To ensure all relevant studies were included in the search outcome, keywords were carefully selected, and each keyword was searched both separately and together with the keyword "COVID-19". Other keywords used were as follows: "cytokine storm"; "plaque control"; "oral care"; "oral hygiene care (OHC)"; "mechanical ventilation"; "intensive care unit (ICU)"; and "ventilator-associated pneumonia (VAP)". Only articles in English were considered. The articles were chosen based on their association with the title of this literature review in terms of their title, relevant abstract and full-text, as long as they were related to the topic and aim of this overview

Cytokine storm and COVID-19

The leading cause of death for patients affected by COVID-19 is respiratory failure, which is consistent with the typical cause of death related to other viruses. This was determined by Chen et al. and reported in their article describing the epidemiological and clinical characteristics of 99 cases of COVID-19 pneumonia in Wuhan, China.⁷ Their descriptions coincide with those of Wang et al.⁸ In order to improve the survival rate of severely affected patients, invasive mechanical ventilation has been recommended. This was suggested by Xie et al. as part of COVID-19 management in China.⁹ Such patients undergo mechanical ventilation until their lungs recover from the injury. Mortality from COVID-19 can be due to other reasons, including septic shock and multi-organ failure. Xie et al. reported that as of February 10, 2020, about 15% of patients in Wuhan developed severe pneumonia and 6% required ventilatory support.⁹ Of those who died before January 30, 2020, approx. 25% received extracorporeal membrane oxygenation (ECMO).⁹ The role of secondary bacterial infection in reducing patients' survival has been mentioned in the literature, including articles by Huang et al.¹⁰ and Liu et al.,¹¹ but the mechanism has not been described clearly. COVID-19 is associated with other complications that may lead to death, including cardiac shock caused by acute myocardial injury, myocarditis and acute kidney injury.^{8,10} Table 1 summarizes the literature on the reported COVID-19 cases.

According to a review by Ragab et al., the innate immune response involves the activity of various inflammatory cytokines and chemokines.¹² Three of the most important cytokines are interleukin (IL)-1, IL-6 and tumor necrosis factor-alpha (TNF- α). They are mainly produced and released by tissue macrophages, mast cells, endothelial cells, and epithelial cells. The term "cytokine storm" is used to describe a sudden acute increase in circulating proinflammatory cytokines, including IL-1, IL-6 and TNF- α , and interferon (IFN) due to the migration of immune cells, such as macrophages, neutrophils and T cells, from the circulation to the site of infection. The sudden increase ultimately results in the destruction of host tissues, brought about by the disruption of the endothelial cell-cell interaction, vascular barrier and capillary damage, and diffuse alveolar injury.

Clinically, a cytokine storm is a life-threatening situation in which the patient requires intensive care due to a high risk of death. Patients experiencing a cytokine storm typically exhibit symptoms of significant systemic inflammation, such as intense fever and hyperferritinemia, hemodynamic instability, and eventually multi-organ failure that can lead to death if not addressed urgently. These symptoms are believed to be the effect of increased levels of circulating proinflammatory cytokines, such as IL-1, IL-6, IL-18, interferon-gamma (IFN- γ), and TNF- α . Acute lung injury is one of the possible consequences, and it may even advance into acute respiratory distress syndrome (ARDS).¹³

Table 1. Literature on the reported coronavirus disease 2019 (COVID-19) cases

Articles	Type of literature	Relevant main findings
Ruan et al. ⁴	letter to the editor on a retrospective multi-center study	<ul style="list-style-type: none"> – The predictors of a fatal outcome in COVID-19 patients included age, the presence of underlying diseases, the presence of secondary infection, and elevated inflammatory indicators in the blood. – The results obtained from this study also suggest that mortality for COVID-19 might be due to virus-activated “cytokine storm syndrome” or fulminant myocarditis.
Chen et al. ⁷	retrospective single-center study	<ul style="list-style-type: none"> – The 2019-nCoV infection had a clustering onset, was more likely to affect older men with comorbidities and could result in severe, or even fatal respiratory diseases, such as ARDS. – The early identification and timely treatment of critical cases of 2019-nCoV infection are important. – Effective life support and the active treatment of complications should be provided to effectively reduce the severity of patients’ condition.
Wang et al. ⁸	retrospective single-center study	<ul style="list-style-type: none"> – Among 138 hospitalized patients with 2019-nCoV-infected pneumonia, the median age was 56 years and 54.3% were men. – Common symptoms included fever (98.6%), fatigue (69.6%) and a dry cough (59.4%). – Chest CT showed bilateral patchy shadows or ground glass opacities in the lungs of all patients. – 36 patients (26.1%) were transferred to ICU due to complications, including ARDS (61.1%), arrhythmia (44.4%) and shock (30.6%). – Patients treated in ICU ($n = 36$), in comparison with patients not treated in ICU ($n = 102$), were older (median age: 66 years vs 51 years) and more likely to have underlying comorbidities (72.2% vs 37.3%), dyspnea (63.9% vs 19.6%) and anorexia (66.7% vs 30.4%). – Of the 36 cases in ICU, 11.1% received a high-flow oxygen therapy, 41.7% non-invasive ventilation and 47.2% invasive ventilation (4 were switched to ECMO).
Huang et al. ¹⁰	retrospective single-center study	<ul style="list-style-type: none"> – By January 2, 2020, 41 admitted hospital patients had been identified as having laboratory-confirmed 2019-nCoV infection. – Most of the infected patients were men (73%), and less than half had underlying diseases (32%), including diabetes (20%), hypertension (15%) and CVD (15%); the median age was 49.0 years (IQR: 41.0–58.0). – Common symptoms at the onset of the illness were fever (98%), cough (76%), and myalgia or fatigue (44%); less common symptoms were the production of sputum (28%), a headache (8%), hemoptysis (5%), and diarrhea (3%); dyspnea developed in 55% of patients, 63% of patients had lymphopenia and all 41 patients had pneumonia with abnormal findings on chest CT. – Complications ARDS (29%), RNAemia (15%), acute cardiac injury (12%), and secondary infection (10%). – In comparison with non-ICU patients, ICU patients had higher plasma levels of IL-2, IL-7, IL-10, GSCF, IP-10, MCP-1, MIP-1α, and TNF-α.
Liu et al. ¹¹	retrospective multi-center study	<ul style="list-style-type: none"> – The majority of patients with 2019-nCoV pneumonia presented with fever as the first symptom, and most showed the typical manifestations of viral pneumonia on chest imaging. – Middle-aged and elderly patients with underlying comorbidities are susceptible to respiratory failure and may have poorer prognoses.

2019-nCoV – 2019 novel coronavirus; ARDS – acute respiratory distress syndrome; CT – computed tomography; ICU – intensive care unit; ECMO – extracorporeal membrane oxygenation; CVD – cardiovascular diseases; IQR – interquartile range; IL – interleukin; GSCF – granulocyte colony-stimulating factor; IP-10 – interferon gamma-induced protein-10; MCP-1 – monocyte chemoattractant protein-1; MIP-1 α – macrophage inflammatory protein-1 alpha; TNF- α – tumor necrosis factor-alpha.

According to a review article by Coperchini et al., ARDS can be defined as the presence of bilateral lung infiltrates accompanied by severe hypoxemia.¹⁴ A cytokine storm can not only result in ARDS, but also in a range of clinical circumstances, such as pneumonia, sepsis, pancreatitis, and the need for a blood transfusion. It has been suggested that the pathogenesis of ARDS involves inflammatory injury to the alveolocapillary membrane, which leads to increased lung permeability and the exudation of protein-rich pulmonary edema fluid into the airspaces, which results in respiratory insufficiency.¹⁵

One of the main causes of morbidity and mortality for COVID-19 patients is the development of ARDS, which consequently leads to low oxygen saturation. The exact mechanism of ARDS in COVID-19 patients is still not fully understood, but reports by Chen et al.,⁷ Huang et al.¹⁰ and Lai et al.¹⁶ suggest that the excessive synthesis and release of proinflammatory cytokines may be one of the key contributing factors. There is further support for this hypothesis, as it has been found that circulating levels of white blood cells, neutrophils, procalci-

tonin, C-reactive protein (CRP), and other inflammatory indices were significantly higher in COVID-19 patients in ICU as compared to non-ICU patients.^{8,17} Another review by Tang et al. proposes that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection activates the innate and adaptive immune responses,¹⁸ which leads to uncontrolled inflammatory responses, and ultimately to a cytokine storm.¹⁹ The review also suggests that SARS-CoV-2 infection not only causes an inflammatory cytokine storm, but that a cytokine storm may lead to ARDS or extrapulmonary multi-organ failure,²⁰ which can result in the exacerbation of COVID-19, or even death.¹⁸ Table 2 summarizes the literature on the cytokine storm related to COVID-19 cases.

Chen et al.,⁷ Wang et al.,⁸ Huang et al.,¹⁰ and Liu et al.¹¹ all reported similar major symptoms of COVID-19 at the initial onset of the disease. These major symptoms include fever, coughing, myalgia/muscle ache, and fatigue. These articles also listed some of the less common symptoms, which include headache, diarrhea, chest pain, nausea, and vomiting. The majority of patients

Table 2. Literature on the cytokine storm related to COVID-19 cases

Articles	Type of literature	Relevant main findings
Ragab et al. ¹²	review	<ul style="list-style-type: none"> – COVID-19 is transmitted via droplets or direct contact, and infects the respiratory tract, resulting in pneumonia in most cases and ARDS in about 15% of cases. – Mortality in COVID-19 patients has been linked to the so-called “cytokine storm” induced by the virus. – The excessive production of proinflammatory cytokines leads to the aggravation of ARDS and widespread tissue damage, resulting in multi-organ failure and death.
Coperchini et al. ¹⁴	review	<ul style="list-style-type: none"> – The so-called “cytokine storm” refers to the uncontrolled overproduction of soluble markers of inflammation, which, in turn, sustains an aberrant systemic inflammatory response; available evidence indicates that a cytokine storm is a major factor responsible for the development of ARDS.
Tang et al. ¹⁸	review	<ul style="list-style-type: none"> – The current evidence shows that severely ill patients tend to have a high concentration of proinflammatory cytokines, such as IL-6, in comparison with those who are moderately ill. – A high level of cytokines indicates a poor prognosis in COVID-19. – The excessive infiltration of proinflammatory cells, mainly involving macrophages and Th17, has been found in the lung tissue of patients with COVID-19 with post-mortem examination. – Recently, an increasing number of studies indicate that a cytokine storm may contribute to mortality for COVID-19. – The review also shows that SARS-CoV-2 selectively induces a high level of IL-6 and results in the exhaustion of lymphocytes.

Th17 – T-helper 17 cells; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2.

(in the case of 1 center, all reported patients¹⁰) developed pneumonia with the evidence of bilateral lung lesions based on various imaging investigations. Acute respiratory distress syndrome was described as one of the complications of COVID-19, with some patients reported to deteriorate quite rapidly. This pattern of deterioration makes it necessary to transfer patients to ICU, where they can receive non-invasive or invasive ventilation as well as ECMO. Patients in the older age range (median (*Me*): 49–57 years) with complications due to underlying systemic conditions, such as hypertension, cardiovascular diseases (CVD) and diabetes, have poorer prognoses. This is consistent with the findings of Yang et al., who documented cases of COVID-19 in China.²¹

Mechanical ventilation and poor oral health

For patients receiving mechanical ventilation, poor oral health is a common issue. This concern was highlighted by Adib-Hajbaghery et al. in an article published in 2013.²² Poor oral health can be a significant concern, as these patients often develop oral health problems in a short period of time due to numerous causes, including malnutrition, the placement of the tracheal tube and the nasogastric tube in the oral cavity, a reduced fluid intake, reduced salivation caused by prolonged mouth opening, and polypharmacy. These reasons were discussed by Kaya et al.²³ Atypical pneumonia has been reported in some cases of COVID-19 and, as suggested by Huang et al., it may be associated with secondary bacterial infection.¹⁰ For this reason, special attention should be given to oral health and periodontal-related diseases in particular, as the latter are commonly associated with Gram-negative pathogenic bacteria, which can potentially complicate the condition of COVID-19 patients.

Due to its distinctive low oxygen tension and pH, which tends to skew toward an acidic environment, as well as the abundance of nutrients in the mouth, the oral cavity is regarded as an ideal microbial incubator. According to Fields, either naturally occurring (e.g., enamel and cementum) or artificially introduced (e.g., dental restorations, fixed or removable prostheses, and implants) hard surfaces in the mouth favor the formation of microbial sediments, which results in the establishment of dental biofilm.²⁴ For reasons mentioned earlier, namely a reduced fluid intake and reduced salivation, dental biofilm is more likely to form and evolve in ICU patients as compared to other patients. This finding was reported in a study by Panchabhai et al. as early as in 2009.²⁵

In a study by Pedreira et al. on children receiving mechanical ventilation over a period of 48 h, Gram-negative bacteria were found to be the prevalent pathogens in their oropharyngeal secretions.²⁶ Since dental biofilm mass increases over time, combined with the accretion of aerobic and anaerobic bacteria and subsequent colonization by predominantly anaerobic Gram-negative bacteria, the presence of dental biofilm may be an essential factor in determining the composition of oral and pharyngeal environment. This was highlighted in a literature review authored by Hillier et al.²⁷ Furthermore, other studies, including that by Rello et al.,²⁸ suggested that biofilm within the oral cavity could be populated by respiratory pathogens, and that the microbes associated with nosocomial pneumonia were found to originate from the oral cavity. Therefore, the presence of poor oral conditions can potentially endanger patients admitted to ICU, especially those undergoing mechanical ventilation.

Studies by Scannapieco et al.²⁹ and Panchabhai et al.²⁵ suggested that some of the bacteria in dental plaque, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, can be major causes of VAP. In fact, Adib-Hajbaghery et al.²² and Haghghi et al.³⁰ examined the impact of oral care interventions on the incidence of VAP among ICU patients undergoing mechanical ventilation and showed positive outcomes for patients that received an intervention.

One of the most common inflammatory diseases in adults is periodontal disease. A study published in 2017, concerning the global burden of the disease, found that severe periodontal disease was the 11th most prevalent condition in the world, making it a significant public health issue.³¹ The burden on healthcare systems is expected to increase as the global population ages, and this may become a significant concern. With advances in periodontal research, there is considerable evidence, such as that presented by Kim and Amar,³² that links periodontal disease with various extraoral and systemic diseases, including type 2 diabetes mellitus, CVD, respiratory tract infections, adverse pregnancy outcomes, and neurodegenerative diseases.

Great interest has been shown in the connection between oral condition, inflammation and systemic diseases, as manifested by many recent studies that have explored these subjects. Microorganisms in the oral cavity not only cause local intraoral inflammation, but also directly contribute to systemic inflammation. A rise in inflammation is the result of toxins being released by microorganisms and the leakage of microbial by-products into the bloodstream. It is vital to acquire more knowledge about the connection between oral and systemic inflammation to better understand the detrimental effects of intraoral inflammation on other organ systems as well as the possibility of increasing the risk of developing non-oral diseases due to oral diseases. This interrelationship and its impact were discussed in a review article by Kim and Amar as early as in 2006.³²

Dental plaque/biofilm as a risk factor for COVID-19 ventilated patients

Paster et al. conducted pioneering research to determine the microbial population in the mouth and found that there are approx. 500–700 prevalent taxa.³³ Oral microbiota, microflora, and microbiome are some of the terms used to describe the intraoral microbial community. These microorganisms have previously been isolated from the saliva, gingival epithelium and other surfaces of the oral mucosa, with the highest density found in dental plaque. As the technology for bacterial detection and profiling improve, more studies are carried out to determine the microbiome of various oral niches, including the various sites of mucous membranes and the tongue, as well as within dental plaque itself. Liu et al. explored the oral microbiome using the most current technique at the time of their study.³⁴ Oral diseases, such as periodontitis and dental caries, are known to be strongly associated with particular bacterial species that are commonly found in the microbial diversity of the dental biofilm. These groups of bacteria, collectively characterized as pathogenic, have progressively been correlated with systemic infections.

There has been interest in exploring the content of the dental biofilm of patients with known systemic symptoms or diseases, as it may provide more information about the connection between the disease of interest and oral microorganisms. This was discussed by Kim and Amar in 2006.³² In a review paper, Gomes-Filho et al. examined the studies which demonstrated that an unhealthy oral cavity was a predisposing factor for respiratory infections, and suggested that oral or non-oral pathogens present in the oral cavity could contribute to respiratory disease.³⁵

As early as in 1997, Garrouste-Orgeas et al. suggested that the colonization of the oropharynx with bacteria is a critical factor in the development of nosocomial pneumonia.³⁶ Munro and Grap discussed this possible mechanism and suggested that the microbes associated with the incidence of VAP might flourish from the center of infection, containing the established habitat for other pathogenic bacteria.³⁷ This is thought to be possible, since the dental biofilm creates a favorable environment for VAP-causing bacteria, which can attach to either tooth surfaces or other microbes present in the biofilm. This enables them to be transmitted from the oral cavity to the lungs, which may lead to the development of VAP. This hypothesis was introduced as far back as in 1992 by Scannapieco et al.,²⁹ and discussed further by Garrouste-Orgeas et al.³⁶ and El-Solh et al.³⁸

Researchers in the past suggested that there was a correlation between periodontal disease, particularly periodontitis, and systemic diseases, including lung diseases or infections. The mechanism for the relation between periodontal disease and respiratory illnesses is still not clear, with the former not always leading to or increasing the incidence of the latter. However, the presence of periodontal disease is thought to change the typical behavior of respiratory illnesses, possibly due to changes in the content and dynamics of oral microorganisms, brought about by the presence of periodontal infection. Weidlich et al. discussed this possible mechanism in their review article published in 2008.³⁹ The microaspiration of oral microorganisms in patients with periodontal infection may have different outcomes when compared to patients without periodontal infection, possibly due to differences in the microbial composition.

Patients severely affected by COVID-19 require mechanical ventilation to increase their chances for survival. The endotracheal tube (ETT) acts as the interconnection between the patient's lungs and the ventilator. The insertion of the tube via intubation is followed by changes in the oral micro-environment and subsequently alters the composition of the oral microbiome. Various studies in the past, including those by Scannapieco et al.²⁹ and Berry et al.,⁴⁰ suggested that changes in the oral environment facilitated the establishment and propagation of respiratory pathogens and other possible opportunistic pathogens that may be present in the oral cavity or inside the pulmonary system itself. How the shift in the content

of the microbiota came about is still not entirely clear. However, it has been suggested that it may be due to the placement of ETT inside the oral cavity and through the trachea, which hinders the removal of dental biofilm, reduces the saliva flow and dries the mucosa due to persistent mouth opening. When managing medical conditions (i.e., treating the condition being the main reason for hospitalization), any medical interventions or even medications given may also contribute to microbial changes.

Retentive factors for dental biofilm

The presence of pathologic changes in periodontal anatomy, such as increased pocket depth, intrabony pockets and furcation involvements, becomes a local contributing factor in the oral cavity that allows dental biofilm to accumulate. Calculus may be present supragingivally, which may encourage the development of the inflammation of the marginal gingiva, or subgingivally, which creates irregular porous surfaces that can act as reservoirs for periodontal pathogens and endotoxins. In a review article, Genco and Borgnakke stated that the presence of subgingival calculus and its plaque layer were associated with the development of periodontitis, with higher rates of disease progression in adolescents and adults.⁴¹

There are other local contributing factors, including poorly completed or deteriorating restorations (i.e., those with overhangs), rough surfaces, subgingival margins, marginal discrepancies, and under- or over-contoured crowns. Fixed appliances like orthodontic braces, crowns and bridges as well as pathological changes, such as endodontic infections, root fractures and cervical root resorption, are all considered facilitating factors that contribute to the accumulation of dental plaque/biofilm. The elimination of most or all (if possible) of these local factors aims to reduce the bacterial load in the oral cavity, which would specifically aid COVID-19 patients by reducing the likelihood of complications like atypical pneumonia.

Oral hygiene for COVID-19 patients undergoing mechanical ventilation in ICU

It has been established that poor oral health may contribute to or complicate some systemic diseases, including respiratory ones. Coker et al. discussed this concept with dependent older adults as a focus of interest.⁴² Various studies, as highlighted by Yurdanur and Yagmur in their review,⁴³ have associated VAP with poor plaque control. The aspiration of oropharyngeal secretions, with their content being influenced by the composition of the dental biofilm, is an independent risk factor for VAP and, according to a systematic review by Berry et al.,⁴⁴ is a key causative

factor for nosocomial infection in ICU patients. A review conducted by Cirillo et al. found that VAP was a complication in 8–28% of patients receiving mechanical ventilation.⁴⁵ Furthermore, original research by Inchai et al. found that a 30-day mortality rate in patients with VAP caused by drug-resistant *Acinetobacter baumannii* was 21.2%, while the rates for multi-drug-resistant, extensively drug-resistant, and pan-drug-resistant *Acinetobacter baumannii* VAP were 31.9%, 56.8% and 66.7%, respectively.⁴⁶

The same systematic review by Berry et al. showed that bacteria accountable for VAP, which may include *Acinetobacter baumannii*, colonize the oral mucosa and are collected in the dental biofilm of intubated patients.⁴⁴ Therefore, providing adequate oral care should be a priority for critically ill patients in ICU, as it is essential in reducing the incidence of VAP. Adequate oral care may not only affect the clinical outcome of these patients, but also improve their overall wellness. This is apparent in the findings of a study by DeKeyser Ganz et al.,⁴⁷ and again highlighted in a review article by Atay and Karabacak.⁴⁸

According to Feider et al., when providing adequate oral care to orally intubated and critically ill patients, the primary goal should be to improve oral hygiene.⁴⁹ This should result in a reduction in the bacterial population in the oropharynx and the dental biofilm, and subsequently reduce the aspiration of the contaminated saliva. This possible mechanism was further discussed by Safdar et al. in their review published in 2005.⁵⁰ Also, according to Coker et al., the practice of adequate oral care for these patients promotes holistic patient care, increases their comfort and prevents halitosis.⁴² The maintenance of oral hygiene with constant adequate oral care is also important to prevent the recolonization of the oral cavity by pathogenic microbes. Table 3 summarizes the literature associating bacterial colonization and VAP.

Mechanical dental plaque control

Dental plaque control for patients on mechanical ventilators is challenging. The main reason is a limited and difficult access to the oral cavity. Evidence also suggests that oral care protocols may not be fully followed by the ICU staff. This is reflected in a survey carried out by Rello et al.,²⁸ involving 59 European ICUs. In a review regarding the clinical effectiveness of the oral health procedures utilized by nurses, performed by Bowsher et al. in 1999, it was found that the sponge Toothette® oral swabs frequently used in ICUs showed limited effectiveness in eradicating the dental biofilm as compared to a regular toothbrush.⁵¹ Therefore, toothbrushes are now thought to be the superior choice in maintaining oral hygiene among ICU patients.

The apparatus for OHC should be carefully chosen based on its benefits, accessibility and potential adverse effects as well as its specific features that set it apart from other

Table 3. Literature associating bacterial colonization and ventilator-associated pneumonia (VAP)

Articles	Type of literature	Relevant main findings
Scannapieco et al. ³⁰	prospective non-randomized study	<ul style="list-style-type: none"> – The oral hygiene of medical ICU patients was poor. – The dental plaque and/or the oral mucosa of 65% of medical ICU patients were colonized by respiratory pathogens, in contrast to only 16% of patients in a preventive dentistry clinic (control). – The potential respiratory pathogens cultured from medical ICU patients included methicillin-resistant <i>Staphylococcus aureus</i>, <i>Pseudomonas aeruginosa</i>, and 10 genera of Gram-negative bacilli.
Garrouste-Orgeas et al. ³⁶	prospective study	<ul style="list-style-type: none"> – Out of 86 ventilated ICU patients, 31 cases of pneumonia were diagnosed (36% of patients). – Oropharyngeal colonization, detected either on admission or from subsequent samples, was a predominant factor for nosocomial pneumonia in comparison with gastric colonization. – Oropharyngeal colonization with <i>Acinetobacter baumannii</i> yielded an estimated 7.45-fold increased risk of pneumonia in comparison with patients not yet or not identically colonized ($p = 0.0004$). – DNA genomic analysis demonstrated that an identical strain was isolated from oropharyngeal, gastric or bronchial samples in all but 3 cases of pneumonia due to <i>Staphylococcus aureus</i>. – The findings provide better knowledge of the pathophysiology of nosocomial pneumonia in mechanically ventilated patients.
Safdar et al. ⁵⁰	review	<ul style="list-style-type: none"> – VAP is the most common nosocomial infection in ICU, and it is associated with major morbidity and attributable mortality. – A major route for acquiring endemic VAP is oropharyngeal colonization by the endogenous flora or by the pathogens acquired exogenously from the ICU environment, especially the hands or apparel of healthcare workers, the contaminated respiratory equipment, hospital water, or air. – The aspiration of the microbe-laden oropharyngeal, tracheal or gastric secretions around the cuffed endotracheal tube into the normally sterile lower respiratory tract results in most cases of endemic VAP. – Strategies to eradicate oropharyngeal and/or intestinal microbial colonization, including chlorhexidine oral care, have been shown (among others) to reduce the risk of VAP.

oral care equipment; for example, its efficacy in plaque removal. Based on the findings of a randomized trial by Needleman et al., it can be concluded that toothbrushes remain the preferred and standard oral care tool as far as dental plaque removal is concerned, as it is recognized that dental plaque is efficiently cleared by means of mechanical interference.⁵² The findings of a study by Yao et al. published in 2011 indicate that an oral hygiene routine consistently carried out twice daily can significantly reduce the incidence of VAP.⁵³ To improve the efficacy of oral hygiene practice, the regularity of care should be determined using daily oral assessments, as suggested by Ames et al.⁵⁴ Conversely, however, a randomized control trial by Lorente et al. found that there was no significant difference in the incidence of VAP in critically ill patients receiving oral care with or without manual toothbrushing.⁵⁵

Electric toothbrush for dental plaque control

As suggested by Verma and Bhat, powered toothbrushes (PTBs) have been promoted as appliances that offer improved biofilm removal capability when compared to manual toothbrushes.⁵⁶ A Cochrane review by Robinson et al. published in 2009 suggested some advantages of PTBs for plaque removal in comparison with manual toothbrushes.⁵⁷ A systematic review and meta-analysis by Elkerbout et al. regarding single brushing exercises analyzed various studies that compared PTBs with manual toothbrushes to investigate their effectiveness in plaque removal and the outcome was in favor of PTBs.⁵⁸ In clinical settings, PTBs were found to be effective in removing

dental plaque, which is supported by a systematic review by Rosema et al.⁵⁹ and a Cochrane review by Yaacob et al.⁶⁰ Powered toothbrushes are also effective in controlling gingivitis. However, research examining the long-term efficacy of PTBs in inhibiting the initiation or progression of periodontitis is limited, as these studies, for instance by Dörfer et al.⁶¹ and Schmalz et al.,⁶² mainly focus on dental plaque, calculus and gingivitis. Needleman et al. concluded that PTBs were exceedingly efficient in removing dental biofilm in intubated ICU patients, and that PTBs should be further evaluated for their potential to reduce the incidence of VAP.⁵² This appliance may also be beneficial in the prevention of lung infections and health complications that are commonly linked to critically ill COVID-19 patients. However, PTBs may not be readily available and their cost may hinder their application as well.

Chemical dental plaque control with chlorhexidine gel

The fundamental instrument used for the removal of dental biofilm is a manual toothbrush. The action of toothbrushing decreases the oral microbial load, which should significantly reduce the risk of lung infections. Toothbrushing combined with chemical plaque control with 0.12% chlorhexidine gel reduces the incidence of VAP, shortens the length of the hospital stay and decreases the ICU mortality rate. These findings are supported by a study by Needleman et al.⁵² and a literature review by Zuckerman.⁶³ The combined use of a regular toothbrush and 0.12% chlorhexidine gel has a synergistic effect on dental plaque, which is evident in various stud-

ies and review papers, including a Cochrane review by Hua et al. published in 2016, which specifically examined OHC for critically ill patients to prevent VAP.⁶⁴ A study by Prendergast et al. emphasized the effectiveness of the combined oral care (which may include the use of a regular toothbrush with chlorhexidine gel) as compared to toothbrushing alone.⁶⁵ It is advocated that the liquids applied for oral care purposes should simultaneously be effective in removing plaque while not causing the irritation of the mucosa or dry mouth. Liquid mixtures that may be used for oral care include chlorhexidine, saline solution and purified water. Studies and review articles by Needleman et al.,⁵² Ames et al.⁵⁴ and Zuckerman⁶³ revealed that even at a relatively low concentration (between 0.12% and 0.2%), chlorhexidine was still effective in preventing VAP.

The establishment and application of a comprehensive OHC program is vital for patients who are at high risk of developing VAP in ICU. It is essential to control and remove dental biofilm from tooth surfaces and the oral cavity. A multi-center study by Ames et al. on the effects of systematic oral care in critically ill patients showed that there was a significant improvement of patients' plaque scores along with a reduction of visible plaque depositions in patients when the system was implemented by critical care nurses.⁵⁴ A proper system or program should incor-

porate the regular and consistent brushing of the teeth, gingiva and tongue, conducted twice daily (minimum), using a small pediatric or single-tufted toothbrush. This implementation is relevant for ICU patients severely affected by COVID-19 with the aim of reducing morbidity and mortality. Table 4 summarizes various types of literature that discusses the effect of oral health on preventing or reducing VAP.

Conclusions

It is essential to re-emphasize the importance of adequate oral care for critically ill patients, especially those on mechanical ventilation, to improve their survival. As bacterial infections have been associated with the atypical pneumonia seen in cases of COVID-19, the control of the oral environment and its microbiome seems like a logical step to be taken for these patients. With the combined measures of toothbrushing and chemical plaque control with 0.12% chlorhexidine gel or other oral care solutions, it is hoped that the incidence of critically ill ventilator-dependent COVID-19 patients contracting atypical pneumonia and/or other infections involving distant organs will be greatly reduced.

Table 4. Literature on the effect of oral care on preventing or reducing VAP

Oral care	Articles	Type of study	Relevant main findings
Chemical only	Panchabhai et al. ²⁵	randomized controlled trial	<ul style="list-style-type: none"> – 512 patients admitted to ICU of an Indian tertiary care teaching hospital were randomized into twice daily oropharyngeal cleansing with 0.2% chlorhexidine (test) or 0.01% potassium permanganate solution (control). – Nosocomial pneumonia developed in 7.1% of patients in the chlorhexidine group and 7.7% in the control group ($p = 0.82$; $RR: 0.93$; $95\% CI: 0.49-1.76$) – During the study period, nosocomial pneumonia developed in fewer subjects (7.4%) than in the 3 months preceding and following the study (21.7%) ($p < 0.001$). – Conclusions: Oropharyngeal cleansing with 0.2% chlorhexidine solution was not superior to oral cleansing with the control solution; however, a decreased incidence of nosocomial pneumonia during the study period suggests a possible benefit of meticulous oral hygiene in ICU patients.
			Kaya et al. ²³
Mechanical only	Yao et al. ⁵³	randomized controlled trial	<ul style="list-style-type: none"> – 53 post-neurosurgical ICU patients were divided into test and control groups. – Both groups received usual hospital care (i.e., daily oral care using cotton swabs). – The test group additionally received a twice daily oral care protocol of toothbrushing with purified water, elevating the head of the bed, and before-and-after hypopharyngeal suctioning. – The control group received mock oral care twice daily (elevating the head of the bed, moisturizing the lips, and before-and-after hypopharyngeal suctioning). – After 7 days of toothbrushing with purified water, the cumulative VAP rates were statistically significantly lower in the test group (17%) than in the control group (71%) ($p < 0.05$). – The test group also had statistically significantly better scores for oral health ($p < 0.05$) and the plaque index ($p < 0.01$). – Conclusions: The findings suggest that as an inexpensive alternative to the existing protocols, toothbrushing twice daily with purified water reduces the risk of VAP, and improves oral health and hygiene.

Oral care	Articles	Type of study	Relevant main findings
Mechanical vs chemical	Needleman et al. ⁵²	randomized controlled trial	<ul style="list-style-type: none"> – Parallel-arm, single-center, examiner- and analyst-masked, randomized controlled trial (N = 46). – Test: PTB; control: sponge Toothette oral swab. – Both were used 4 times a day for 2 min and patients received 20 mL of 0.2% chlorhexidine mouthwash at each time point. – The results showed a low prevalence of respiratory pathogens throughout the study period, with no statistically significant differences between the groups. – A highly statistically significantly greater reduction in dental plaque was obtained with the use of PTB in comparison with the control treatment ($p = 0.006$). – The total viable bacterial count was also highly statistically significantly lower in the test group at day 5 ($p = 0.002$). – Conclusions: PTBs are highly effective for plaque removal in intubated ICU patients and should be tested for their potential to reduce the incidence of VAP and health complications.
	Lorente et al. ⁵⁵	randomized clinical trial	<ul style="list-style-type: none"> – Randomized clinical trial in medical-surgical ICU (N = 436). – Test: oral care including 0.12% chlorhexidine digluconate with toothbrushing; control: oral care including 0.12% chlorhexidine digluconate without toothbrushing. – There were no statistically significant differences between the groups regarding the incidence of VAP (9.7% –with toothbrushing vs 11.0% – without toothbrushing) ($p = 0.75$). – Adding manual toothbrushing to chlorhexidine oral care does not help prevent VAP in critically ill patients on mechanical ventilation.
Mechanical and chemical	Berry et al. ⁴⁰	randomized comparative study	<ul style="list-style-type: none"> – Single-blind, randomized comparative study involving adult ICU patients in a university hospital (N = 109). – All patients underwent cleaning with a toothbrush and a non-foaming toothpaste. – Group A (control): additional hourly oral rinse with sterile water; group B (test): additional hourly sodium bicarbonate mouthwash; group C (test): twice daily irrigations with chlorhexidine 0.2% aqueous oral rinse and additional hourly irrigations with sterile water. – No significant differences were found between the groups for all clinical data. – Group B showed a greater trend toward a reduction in bacterial colonization; however, no significant differences could be demonstrated at day 4 of admission ($p = 0.302$). – The incidence of VAP was evenly spread between groups B and C (5%), while in group A, it was only 1%. – Conclusions: While a number of studies have advocated the use of various mouth rinses in reducing the colonization of dental plaque, a standardized oral hygiene protocol that includes the use of mechanical cleaning with a toothbrush may be a factor reducing the colonization of dental plaque with respiratory pathogens.
Mechanical and/or chemical	Hillier et al. ²⁷	review	<ul style="list-style-type: none"> – No consensus on best practice for oral hygiene in patients being treated with mechanical ventilation has been found. – Although chlorhexidine is the most popular oral care product, no consensus has emerged on its concentration or protocols for oral care. – The implementation of an oral care protocol, ongoing nurse education and evaluation are important in reducing the incidence of VAP.
	Zuckerman ⁶³	review	<ul style="list-style-type: none"> – Oral health has been shown to greatly contribute to the development or prevention of VAP; it can be compromised by critical illness and mechanical ventilation while being influenced by nursing care. – Oral health is managed by proper oral care with the use of oral chlorhexidine in order to decrease oral bacteria and the potential oropharynx colonization. – There is a decrease in the VAP rates with the use of oral interventions, such as chlorhexidine. – The research results support and influence patient care practices, considering that nursing and medicine are driven by evidence rather than experience to prevent avoidable patient harm.
	Hua et al. ⁶⁴	review	<ul style="list-style-type: none"> – OHC, including chlorhexidine mouthwash or gel, reduces the risk of developing VAP in critically ill patients from 24% to about 18%; however, there is no evidence of a difference in the outcomes with regard to mortality, the duration of mechanical ventilation or the duration of the ICU stay. – There is no evidence that OHC including both antiseptics and toothbrushing is different from OHC with antiseptics alone. – Some weak evidence suggests that povidone iodine mouth rinse is more effective than saline/placebo, and saline rinse is more effective than saline swab in reducing VAP. – There is no sufficient evidence to determine whether PTBs or other oral care solutions are effective in reducing VAP. – There is also insufficient evidence to determine whether any of the interventions evaluated in the studies are associated with adverse effects.

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Preventing the suspension of dental clinics by minimizing the risk of SARS-CoV-2 transmission during dental treatment

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Abstract

Due to a high risk of the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during dental work, the coronavirus disease 2019 (COVID-19) pandemic has had a considerable influence on the functioning of dental clinics. The elevated risk of transmission is related to the production of aerosol containing secretions from the upper respiratory tract that is produced during dental procedures.

The purpose of this narrative review was to present the current knowledge concerning COVID-19 and to propose methods for reducing the spread of the virus. Dental staff should follow the current guidelines and ensure safety at work through the use of personal protective equipment (PPE), including FFP2/FFP3 filter masks, the implementation of the screening protocols and telephone consultations as well as the appropriate preparation of dental practices and patient waiting areas. In addition, it is essential to reduce the number of people simultaneously occupying one building by effectively planning visiting times or discouraging patients from accompanied visits. Procedures that may contribute to production of the aerosol should also be limited.

Keywords: aerosols, COVID-19, dental staff, dentists, personal protective equipment

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic was officially declared by the World Health Organization (WHO) on March 11, 2020.¹ No previous epidemics, including those caused by coronaviruses such as severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) or Middle East respiratory syndrome coronavirus (MERS-CoV), have had such a wide-ranging impact on the economy, and on the functioning of dental clinics and other healthcare facilities.

The SARS-CoV-2 virus has been the subject of many studies and, to this day, we do not know the long-term effects of the infection, which appear to be very diverse.² According to WHO reports, the most common symptoms of infection are increased body temperature, fatigue and a dry cough. Less often, there are instances of loss of taste and/or smell, nasal congestion, conjunctivitis manifested by the redness of the eyes, muscle and joint pain, sore throat, headache, skin rash, diarrhea, nausea and vomiting, chills, and dizziness. Patients with a severe COVID-19 infection may also experience confusion, the loss of appetite, or persistent chest pain or tightness.³ Studies have shown that the severity of the disease and the risk of mortality are influenced by the patient's age (over 65 years), the presence of pre-existing diseases and obesity.^{2,4–8} Coronavirus disease 2019 can also affect the whole body, including the parameters of the coagulation system. For example, studies have outlined the possible appearance of disseminated intravascular coagulation (DIC), even in patients with a congenital hemorrhagic diathesis.¹⁰ Laboratory studies of patients with SARS-CoV-2 infection have shown an increase in prothrombin time, a decrease in platelet counts, an elevation in fibrinogen levels, a rise in D-dimer levels, and increases in both coagulation factor VIII and von Willebrand factor.^{8,11–14}

A special role in the transmission of SARS-CoV-2 is played by the aerosol produced during dental procedures, containing secretions from the upper respiratory tract. The virus itself can be transmitted by both symptomatic and asymptomatic individuals.¹⁵ Due to the character of dental work, dental clinics can be a place of a high risk of the transmission of the virus. The following review is narrative in nature, and aims to present information on viral transmission in dental clinics and to summarize the guidelines for dental treatment during the COVID-19 pandemic.

Material and methods

The criteria for the inclusion of studies and articles in the present review are listed below. The review has a narrative structure; it was conducted by 4 researchers and is limited to studies related to oral hygiene, dental care, the SARS-CoV-2 virus, and the functioning of dental clinics

in the era of the SARS-CoV-2 pandemic. The studies included in the analysis concerned guidelines, recommendations, case reports, and academic work, regardless of the original language, year and status of the publication, as long as there was sufficient evidence provided and the studies were related to the subject. The PubMed/MEDLINE database was used to obtain information from the National Library of Medicine (NLM) based on 2 search strategies. One strategy used Medical Subject Headings (MeSH), whereas the other one referred to the keywords in the text. The search involved items published up to November 21, 2020. When a more directed search was needed, related articles were evaluated. Recommendations from international organizations, such as WHO, the Polish Dental Society (PTS), the American Dental Association (ADA), the Royal College of Surgeons of England, the World Federation of Hemophilia (WFH), and the Centers for Disease Control and Prevention (CDC), were also examined. In addition, the bibliographic references in all of the searched reviews and studies were screened for additional research reports. Due to the limited number of original articles, and the lack of randomized and quasi-randomized studies published in this field prior to the end date of the search process, the results were presented in the form of a narrative review. The aim was to compile studies, guidelines and case reports, and make it easier for dentists to perform procedures and work in the rapidly changing epidemiological situation. Systematic presentation of all data is necessary due to the ongoing COVID-19 pandemic and the risk of the transmission of the virus during dental treatment. Eventually, 40 articles and 8 websites were included.

Discussion

The SARS-CoV-2 pandemic has had a great impact on access to dental care and on dental services.^{16,17} According to a study conducted at the Medical University of Silesia in Katowice, Poland, during the government lockdown in the spring of 2020, as many as 71.2% of dentists decided to suspend clinical practice.¹⁶ The main reasons for the cessation of work were insufficient access to personal protective equipment (PPE) and subjective feelings about COVID-19, including a sense of anxiety and uncertainty. In addition, a decrease in the number of patients in dental clinics was observed as compared to the period before the pandemic was announced.¹⁶ Reducing the number of follow-up visits and the postponements of the scheduled treatment can have a sizable impact on the oral health of the population, thus contributing to an increase in the number of patients with acute periapical tissue inflammation, abscesses or acute pulp inflammation.^{17,18}

Scientific research confirms that there is a high risk of virus transmission in dental clinics.^{7,16,19} This is related to the performance of numerous procedures leading to

the formation of aerosol that contains microorganisms, drops of saliva, blood, water, and other contaminants.²⁰ In addition, close contact between the dentist and the patient promotes the transmission of potential infection and causes dental staff to be a source of cross-transmission.²¹ National and international organizations, such as ADA, the Royal College of Surgeons of England, CDC, and PTS, are monitoring and evaluating the spread of the virus on an ongoing basis as well as constantly providing recommendations and guidelines on the functioning of dental practices. According to the current epidemiological situation, it is important to monitor and adapt dental clinics to the latest guidelines.

Many international associations state that the primary way to reduce the risk of the transmission of the virus is through the use of PPE. Surgical masks do not provide full protection against the inhalation of infectious agents, but constitute a barrier between mucous membranes and the sprayed drops.²⁰ The best respiratory protection would be the use of masks with a filter. These are marked with symbols N95, N99 and N100 according to American standards, or FFP1, FFP2 and FFP3 according to European classifications. The quality of a filter marked with the N95 standard is assumed to be nearly equal to that of a filter marked with the European symbol FFP2.²¹ Based on the analysis of scientific studies and recommendations, authors encourage the use of masks of at least N95 or FFP2 standard. In the case of a mask with an exhaust valve, an additional mask is needed to cover the valve.²⁰ Researchers also point out that fabric face guards do not constitute individual protection.²⁰ Additionally, glasses/face shields and medical clothing are also advised as part of PPE, and it is suggested to use a long-sleeved cover under the gloves to protect the forearms.²¹

It should be noted that SARS-CoV-2 can persist on surfaces such as stainless steel or plastic and can remain infectious for up to 3 days at room temperature.²² Therefore, frequent surface and air disinfection is of vital importance in preventing infection. Items in the waiting room that are not suitable for regular cleaning and sterilization, such as magazines or toys, should be removed from the room.^{20,23} For coronaviruses, it is preferable to use a product containing 62–71% ethanol, 0.1% sodium hypochlorite or 0.5% hydrogen peroxide for 1 min.²⁴ Hand hygiene should be carried out with soap and water, followed by the application of a product containing 70–90% alcohol.²¹ Hand sanitizers, together with instructions for use, should also be provided to patients before entering a medical room.²⁰ Air disinfection also plays an essential role in the fight against the pandemic. An interesting solution seems to be the use of ozone equipment in dental offices between visits or in the waiting room after work. Plasma devices may also be used, as they are characterized by a high level of disinfection capability.²¹ Modern technologies can also be helpful in reducing the number of bacteria, viruses and fungi on flat surfaces. High effica-

cy has been demonstrated with the use of hydrogen peroxide fumigation.²¹ Depending on the financial capacity of the clinic, dentists should consider the daily use of disinfection devices. It is crucial to follow the recommendations of the device manufacturers, and it should be noted that some methods of disinfection can affect the quality and durability of various items in dental clinics. In addition, the specific methods may vary, for instance, in the time of room ventilation or the length of the disinfection process. When ultraviolet (UV) lamps are applied, surface disinfection occurs 8 h after switching the device on and it is recommended to leave the room during this time.²⁵ A shorter operating time is needed for ozone generators; depending on the room size, ozone disinfection takes an average of 4–5 h. However, it is essential not to apply other disinfection equipment, including UV lamps, at the same time.²⁵ The main disadvantages of ozone generators are the possibility of causing damage and discoloration to plastic or rubber components as well as the inadequate selection of the ozone dose for the room size.²⁵ When ozone disinfection is completed, it is recommended to ventilate dental offices and avoid entering the rooms for up to 2 h after the process.²⁵ Significantly shorter ventilation times (30 min on average) and disinfection times (about 10 min) are possible with fumigators.²⁵

Until the end of the acute phase of the pandemic, recommendations also include the admission of only urgent patients to the dentist's office.²⁶ In any case, it is necessary to assess the risk associated with the postponement of treatment and the risk associated with the possible transmission of the virus.²⁰ It is suggested to consider providing telephone consultations, and the possibility of preparing electronic consents and prescriptions. Additionally, telephone consultations should be recorded in the patient's medical records.²⁷ According to a study published in April 2020 by WHO, concerning the use of analgesics, there is no evidence to indicate any influence of non-steroidal anti-inflammatory drugs (NSAIDs) on the occurrence of serious adverse events, long-term survival or quality of life of patients with confirmed COVID-19.²⁸ The previous WHO statement, prior to this study, discouraged the use of NSAIDs in this group of patients. However, it should be noted that there are particular diseases where these drugs are contraindicated. It refers to patients with congenital hemorrhagic diathesis, as NSAIDs affect platelet aggregation.²⁹ In such conditions, the approved analgesic is paracetamol (acetaminophen) at a dose not exceeding 60 mg/kg/day or 3 mg/day. The American Dental Association issued recommendations to help determine what constitutes an emergency case.³⁰ This classification system, based on the time required to intervene, divides emergency situations into 3 categories (Table 1). Other kinds of dental treatment that are not listed in the table, such as preventive or hygienic procedures, are regarded routine treatment that can be delayed. Numerous publications recommend that the appointment at the doctor's

Table 1. Modified dental emergency classification by the American Dental Association (ADA)³¹

Classification of emergencies	Purpose of the intervention	Symptoms
Dental emergencies	immediate treatment	<ul style="list-style-type: none"> – uncontrolled bleeding – cellulitis/diffuse soft tissue bacterial infection with intraoral or extraoral swelling that potentially compromises the patient's airway – trauma involving facial bones, potentially compromising the patient's airway
Urgent dental care	relieving severe pain, reducing the risk of infection and reducing the burden on hospital emergency departments	<ul style="list-style-type: none"> – severe dental pain from pulpal inflammation – pericoronitis or third-molar pain – surgical post-operative osteitis, dry socket dressing changes – abscess – localized bacterial infection resulting in localized pain and swelling – tooth fracture resulting in pain or causing soft tissue trauma – dental trauma with avulsion/luxation – dental treatment required prior to critical medical procedures – final crown/bridge cementation if the temporary restoration is lost, broken or causing gingival irritation – biopsy
Other urgent dental care	–	<ul style="list-style-type: none"> – extensive dental caries or defective restorations causing pain – suture removal – denture adjustment in radiation/oncology patients – denture adjustment or repairs when the masticatory function is impeded – replacing temporary fillings in endodontic access openings in patients experiencing pain – snipping or adjustment of an orthodontic wire or appliances piercing or ulcerating the oral mucosa

office should be made by telephone in order to verify the reasons for a visit and to apply a screening protocol.^{20,23} The authors of this article support this opinion and state that the selection of patients by phone may also enable the gathering of detailed information on dental problems and oral health. On the basis of personal experience and the recommendations of international societies, it is suggested to obtain any information regarding the patient's close contacts with any persons tested positive for COVID-19 or being under quarantine. Moreover, it is advisable to collect information regarding the presence of symptoms suggestive of respiratory infections (cough, elevated body temperature, shortness of breath) or travel abroad to high-risk areas.^{7,15,19,21,27,31} Of note, the confirmation of a negative test result for SARS-CoV-2 does not relieve medical personnel of extreme caution and PPE should be applied in all cases. Patients with confirmed COVID-19 should be referred to a treatment center designated for receiving patients from this group^{17,31} or, if possible, it is recommended to postpone dental treatment until isolation or quarantine is completed.²⁰ Furthermore, it is important to adequately space appointments to allow time for full cleaning and disinfection after dental surgery,²¹ and to minimize the number of patients in the waiting room.^{20,23} In addition, it is suggested to place the seats in the lobby in such a way as to maintain the recommended social distance between individuals.²⁰ This will not only decrease the risk of viral transmission, but may also have a positive impact on the patients' sense of safety, as it has been shown that the highest risk of the transmission of COVID-19 infection is associated with close contact with another patient.³²

In order to minimize the risk of the transmission of the virus in the dental clinic, the patient should be asked to attend alone or only with a person necessary to carry out

the visit,^{20,23} such as a legal guardian in the case of a child or an incapacitated person. The patient should be informed that, when entering a medical institution and during screening, both he/she and any accompanying person are obliged to wear a mask that covers the mouth and the nose.^{20,23} It is the responsibility of the healthcare professional to ensure that each entering person complies with the recommendations and to conduct a test for symptoms (including the measurement of body temperature) consistent with SARS-CoV-2 infection.^{20,23} It is important to document the lack of symptoms and, in the case of elevated body temperature with the absence of other general symptoms associated with COVID-19, to draw attention to any possible association with a dental diagnosis.²⁰

In the dental clinic, before examination, the patient should be instructed to rinse their mouth for a minimum of 30 s with a disinfectant-based preparation. The proposed solutions are 0.2% chlorhexidine, 2% Listerine[®], 2% iodine povidone, or 0.5–1% hydrogen peroxide.^{7,21,31} In addition, to eliminate viruses, it is advised to consider the use of modern technologies, such as the ozone therapy or the laser therapy. The neodymium-doped yttrium aluminum garnet (Nd:YAG) laser (1,064 nm) has been shown to have an antibacterial effect that is dependent on the thermal warming of the bacterial environment and local heating inside pathogens.³³ These properties are used in dentistry primarily in the sterilization and disinfection of root canals. In addition, the Nd:YAG laser is the most recommended laser in endodontics. Studies show that the application of the Nd:YAG laser reduces the number of *Enterococcus faecalis* and *Escheria coli* bacteria by 99.92%.³³ During this particular laser therapy, it is important to use a fiberglass tip with a diameter of approx. 200 µm, placed about 1–2 mm from the apex. The tip should be moved toward the crown with circu-

lar and gradual movements. The laser is used 4 times for 5–10 s with 20-second breaks. Safe parameters for the Nd:YAG laser are 100 mJ, 15 Hz and 1.5 W.³³ Another method for tissue disinfection in the mouth is the ozone therapy in the form of ozonated water or oil.³⁴ The use of ozone water reduces the number of *Streptococcus mutans* bacteria in the plaque, and thus contributes to the prevention of the progression of caries.³⁴ Moreover, ozone can also be used as an additional therapy to disinfect root canals and treat gingivitis, periodontitis, or even osteonecrosis.³⁴

The SARS-CoV-2 virus has been identified in the airway,³⁵ saliva³⁶ and gastrointestinal tract.^{36,37} For this reason, it is not recommended to take intraoral X-rays,^{7,30} as it can provoke a gag reflex or increase salivation. If a radiological diagnosis is required, extraoral imaging, such as pantomographic X-ray or cone-beam computed tomography (CBCT), is preferred. It is permissible to take an intraoral photograph, but the dentist should choose bitewing radiography. If the doctor decides to take a dental photograph without a positioner, the patient should disinfect their hands before holding the X-ray film.²¹ If a positioner is used, it must be sterilized after each use.²¹ When taking intraoral photographs, it is suggested to use a double cover on the radiological sensor to reduce the risk of contamination.¹⁵ Avoiding small-format pictures refers primarily to patients with confirmed SARS-CoV-2 virus infection.³⁸ In such cases, intraoral radiograms should be used in patients who cooperate and can breathe well through the nose. In order to control the risk of the transmission of the virus, the patient should be informed of a possible gag reflex and asked about previous problems with taking an intraoral photograph.³⁸

Patients who report to dental clinics during a pandemic are primarily patients in pain. Additionally, patients with concomitant diseases deserve special attention. In situations where urgent intervention is necessary, patients with congenital hemorrhagic disorders are particularly difficult cases, as a pre-treatment consultation with a hematologist is often necessary. Extraction is a procedure involving the risk of delayed or prolonged bleeding.^{39–41} In this case, it should be remembered that an alternative is endodontic treatment.⁴² During such a procedure, it is essential to use an apex locator and to be especially aware of work duration times.⁴² This can prevent possible bleeding from periodontal fibers. The proposed dressing used between visits is calcium hydroxide, which has drying properties.^{42,43} In the presence of residual vital pulp in the bleeding canal, the rinsing protocol should be supplemented with sodium hypochlorite solution.^{39,40} Procedures requiring extreme caution and the supplementation of the missing factor are mandibular nerve block anesthesia and infiltration anesthesia from the lingual side.^{43,44} Intrapulpal, interdental or infiltration anesthesia from the buccal side and mental nerve block anesthesia can be considered safe.⁴⁵

The scheduled visits, such as prosthetic and orthodontic treatment, should be postponed until the end of the acute phase of the pandemic.²⁶ In order to resist the transmission of the virus, it is recommended to use rubber dams^{7,15,23,31} and to apply dental suction²³ whenever possible. Authors recommend paying attention to the type of suction device, as this directly contributes to the amount of aerosol formed in the doctor's office. Obtaining the smallest possible amount of aerosol in the environment requires the use of efficacious suction equipment.⁴⁶ During some procedures, such as the removal of calculus, it is worth considering to replace mechanical tools with hand instruments.²³ When working with children, the use of atraumatic or minimally invasive procedures should be considered.⁴⁷ An interesting alternative to the traditional cleaning of a dental cavity with rotary tools is a laser. Authors suggest, particularly in the time of the pandemic, that laser application may be of particular importance. While using the Er:YAG laser with the H14 handle (Light-Walker; Fotona, Ljubljana, Slovenia), with the parameters set at 300 mJ energy, 20 Hz frequency and 6 W power, and water/air coolant 6/4, less aerosol is produced as compared to the traditional tooth preparation procedures.⁴⁶ In order to minimize the risk of vomiting during impression-taking procedures, and thus to reduce the transmission of SARS-CoV-2, it is suggested to carefully adjust the proper dental tray before taking the impression,^{19,48} to avoid the use of products of excessively fluid consistency²¹ and to ensure the correct position of the patient⁴⁸; for patients with a developed gag reflex, it is advisable to use a local anesthetic.⁴⁸ Special care should be taken if bleeding in the mouth occurs, as the swallowed or inspired blood may cause vomiting or coughing.²¹ Furthermore, the computer-aided design/computer-aided manufacturing (CAD-CAM) technology is extremely useful today, and it can replace traditional impressions in the future.⁴⁸

Conclusions

The current COVID-19 pandemic has introduced many restrictions at the individual and social level. The problem has become global and has posed new health and dental challenges. Doctors and dentists are not only obliged to pay attention to the general health of the patient, but also to their own safety and protection against the SARS-CoV-2 virus. Prevention and dental education, which help to maintain oral health, should become essentials, as they minimize the need for dental surgeries and reduce the number of emergency procedures. In the case of an urgent dental intervention, dentists must follow all applicable safety rules to protect themselves, the patient and the dental clinic area. It is also suggested to provide treatment during a single dental visit, while taking preventive measures to reduce the number of consecutive visits. Moreover, it is also important to conduct a detailed

medical and dental interview, and to manage documentation. Efficacious communication and information flow can be very helpful in preparing the right treatment plan. Given the current situation, every action should be carefully planned and thought out. Oftentimes, the dentist will have to assess the risks associated both with the discontinuation of treatment and with the potential infection with the SARS-CoV-2 virus. In this way, together we can limit the spread of the pandemic.

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Occupational health in oral radiologists: A review

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Abstract

Work is a fundamental axis for the development of societies and human well-being, but if a person cannot adapt to their work area and work environment, the individual may be affected by occupational or co-existing illnesses that get exacerbated when working.

A scientific search was conducted in the main health databases – MEDLINE (via PubMed), Web of Science, SciELO, Scopus, Google Scholar, and Dialnet – using the keywords “occupational health”, “occupational diseases”, “occupational accidents” AND “oral radiology” OR “oral radiologists”. Systematic reviews as well as observational, cross-sectional and longitudinal studies were included. Case reports, letters to the editor, editorials, and opinion articles were excluded. In total, 496 articles were recovered, and only 51 fulfilled the selection criteria. Signs and symptoms that affect oral radiologists include back pain, shoulder pain, wrist pain, tenosynovitis, computer vision syndrome (CVS), stress, depression, and burnout syndrome. Preventive occupational health (OH) measures are proposed to help eliminate or alleviate the symptoms associated with non-ergonomic habits at work. Oral radiologists are exposed to several risks and occupational diseases inherent and/or related to their work. By implementing simple habits and ergonomic advice, well-documented in the literature, these risks can be avoided.

This review aimed to provide scientific information on the current concepts of OH in oral radiologists in order to help prevent occupational diseases and occupational accidents, and guarantee safe professional practice.

Keywords: occupational health, ergonomics, radiology, dental staff

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Introduction

Work plays an especially important role in the development of human societies. As knowledge and technology advance, the forms, modes and environments of work also advance.¹ It has been shown that the health of workers is conditioned by different social and individual factors as well as by access to health services, and the forms, modes and environments of work.^{2,3}

Occupational health (OH) has been defined by the Pan American Health Organization (PAHO) as the promotion and maintenance of the highest degree of physical, mental and social well-being of workers in all occupations by preventing deviations from health, controlling risks, and adapting work to people and people to their work positions.³⁻⁵ Since OH is based on the law, each country has its decrees, laws and regulations, created according to the guidelines of the United Nations (UN), the European Union (EU) and the Organization of American States (OAS), which are major international mainstays for countries.^{4,6,7}

The International Labor Organization (ILO) has classified occupational diseases into the following groups: those caused by chemical, physical or biological agents; infectious or parasitic diseases; respiratory diseases; skin diseases; musculoskeletal disorders; mental and behavioral disorders; occupational cancer; and diseases caused by other substances and agents, not included in any of the abovementioned sections. There are also other diseases, not included on this list, that may be related to work activities.^{5,7-9}

Oral radiology is a discipline that involves the diagnosis of the diseases and disorders of the mouth, face and jaws with the use of X-rays and other types of radiological techniques. Apart from diagnosing a disease, radiological examinations enable the development of treatment plans and the monitoring of the evolution of lesions and the disease over time.^{6,10-14} This profession presents many inherent risks related to the occurrence of diseases and work-associated accidents, which may imply immediate or long-term problems. Moreover, an important aspect to consider is that health professionals, including oral radiologists, live in a competitive environment that is highly demanding and seeks perfection in their daily work; this leads to high levels of emotional stress.¹⁵⁻¹⁸ In addition, the impact of the coronavirus disease 2019 (COVID-19) pandemic on mental health, and consequently on the professional practice of the whole medical staff, must be taken into consideration.¹⁹⁻²¹

This review evaluates the discomfort, illnesses and occupational problems derived from the practice of oral radiology, and proposes some ergonomic recommendations.^{1,3,7,8,14} Further, the purpose of this review was to provide scientific information on the current concepts of OH in oral radiologists in order to help prevent occupational diseases and occupational accidents, and guarantee safe professional practice.

Methodology

A literature search was carried out using the main sources of scientific information, such as MEDLINE (via PubMed), Web of Science, SciELO, Scopus, Google Scholar, and Dialnet, and search terms with a date limitation of the last 10 years to include information regarding OH and oral radiology. The keywords used were “occupational health”, “occupational diseases”, “occupational accidents”, “ergonomic”, “oral radiology”, and “oral radiologists”. Systematic reviews as well as observational, cross-sectional and longitudinal studies were included. Case reports, letters to the editor, editorials, and opinion articles were excluded. Thus, 496 articles were recovered, of which only 51 fulfilled the selection criteria. These studies were included to evaluate the development of theoretical topics. The bibliographic search was carried out until December 5, 2020 (Fig. 1).

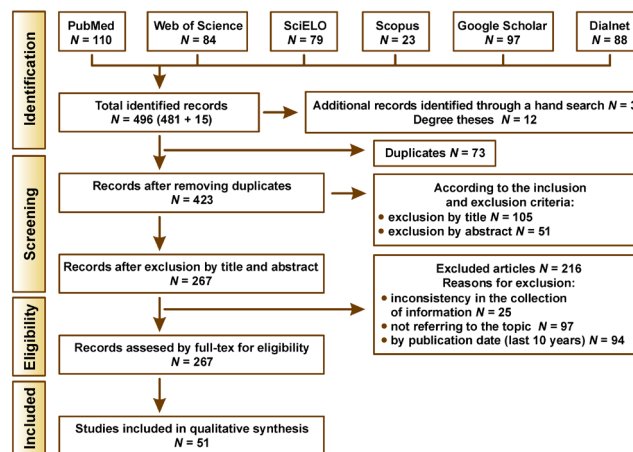


Fig. 1. Flow chart for collecting information

General aspects of occupational health

In recent decades, globalization, technology, the diversification of societies, demographic changes, and the overcrowding of cities have exerted a strong impact on the workplace, leading to the concept of work ethics. This concept is important, since it seeks to balance the risks and benefits between the interested or contracting parties, which, in this case, involve employees and employers.⁷

Work is linked with health and can affect not only physical health, but also mental health due to the transfer of workload to home or other personal spheres. Epidemiological studies have shown that the morbidity and mortality of a population are closely related to labor demands. Occupational accidents due to inadequate working conditions, or worker or employer negligence, can not only have strong repercussions on health, but can also end life.^{8,9}

Access to health services adds to the quality of life of populations, families and workers. A study carried out in 17 countries of EU evaluated the health systems of each country and reported that while worker healthcare was mandatory, annual controls to assess the work capacity of workers, and human, institutional and infrastructure resources were insufficient.^{10,11}

Occupational diseases in oral radiology

Work-related musculoskeletal symptoms refer to muscular, nerve, cartilaginous, bone, tendon, vascular, or joint-related discomfort in various parts of the body and the human support system that are mainly attributable to the work environment and increasingly worsen over time. Over time, musculoskeletal complaints can become long-term degenerative disorders that decrease productivity at work.¹²

In the study by Al Shammari et al, the most prevalent discomfort reported by radiologists was lumbar pain, pain in the upper extremities and the carpal tunnel, and tenosynovitis.¹³ Other studies described pain in the lower back and the spine as well as the neck, as repetitive stress injuries.^{14,22} According to various studies, radiologists most frequently report pain or discomfort at least once a week in the neck (66%), the lower back (61%), the upper back (43%), the right shoulder (36%), and the right wrist (33%).^{13,23}

Stress is becoming more and more common in the workplace. It has been reported that 55% of radiologists experience symptoms related to stress and exhaustion. Stress often results in physical and emotional consequences, including cardiovascular problems, the lack of sleep, and in extreme cases, severe psychological disorders. The associated risk factors for stress are feeling undervalued, feeling belittled, the inability to achieve goals, experiencing barriers that prevent the person from performing their work and achieving personal development, working conditions, unfair work rules, and loneliness, which combined, can generate synergy.^{24,25}

Clinical depression can also affect radiologists, with the most common diagnostic criteria being a depressed mood most of the time, the loss of pleasure, the loss of interest in most of the daily activities, slowing down in carrying out daily activities, a decreased appetite, an unjustified weight loss, permanent insomnia or hypersomnia, restlessness, fatigue and tiredness almost every day and all day, a decreased ability to think, concentrate or make decisions, feelings of guilt and low self-worth, and thoughts of death (the fear of death or suicidal thoughts). A professional is defined as having depression with the presentation of 5 or more of these criteria for a period of 2 or more weeks.^{26,27}

Burnout syndrome involves chronic work stress that affects a person in all facets of their life, both in the work environment and outside. Some of the consequences of this

syndrome are low self-esteem, low energy, the inability to fall asleep, permanent fatigue, weakness, a feeling of frustration, negative attitudes, increased irritability, the degeneration of social relationships, the loss of motivation with regard to life affairs, and depression.²⁸ This syndrome has 3 components: emotional exhaustion, a feeling of frustration, and a negative attitude toward oneself and others.²⁹ In South America, the stress levels associated with burnout syndrome are low among radiologists^{30,31}; however, high levels of burnout have been reported in Canada, both in apprentices and professionals.^{32,33} On the other hand, in India, 54% of radiologists report repetitive stress injuries, 52% of which are accompanied by pain in the neck.³⁴

Computer vision syndrome (CVS) has been defined by the American Optometric Association (AOA) as a set of ocular and visual problems related to activities involving near vision, and is experienced while using computers.³⁵ The manifested symptoms are headache, difficulty in focusing, dry eyes, red eyes, irritated eyes, double vision, and blurred vision, all of which are strongly related to the number of hours in front of a screen. Among Canadian radiologists, the prevalence of CVS is 36%.^{36–38}

Metabolic syndrome describes several conditions that include hyperglycemia, high triglyceride and cholesterol levels, obesity, and hypertension. These parameters are found to be higher in professionals with a higher degree of work stress. It has been described that 7.1% of radiologists have metabolic syndrome, presenting 3 or more simultaneous pathological metabolic abnormalities.³⁹ More scientific evidence on metabolic syndrome among radiologists is required.

Despite the advent of digital radiology, the use of analog radiology is still common today in many parts of the world. Therefore, the working conditions and the substances used for image processing can affect the health of radiologists. Some of the symptoms described include headaches, a sore throat, a shortness of breath, skin rash, a bad taste in the mouth (chemical taste), a runny nose, itchy eyes, nausea, asthma, fatigue, watery eyes, a persistently stuffy nose, a persistently itchy nose, and sneezing, which are collectively known as “darkroom disease”.⁴⁰

Additionally, an important aspect to consider is that health professionals, including oral radiologists, have been negatively impacted by the COVID-19 pandemic. The disease has had a significant effect on mental health of the whole medical staff, and consequently on professional practice.^{19–21} Therefore, a good, healthy and comfortable work environment is required.

Ergonomics in dental radiology

The International Ergonomics Association (IEA) defines ergonomics as the scientific discipline of studying the individuals’ relationships, interactions and adapta-

tions with/to the work environment and each of the elements that compose it, in order to guarantee human well-being and the productivity of the system, to achieve maximum efficiency in terms of the use of movements, space and time, to minimize physical and mental stress, and to prevent occupational diseases, which can be very difficult to treat once they appear.⁴¹

In the work setting, equipment must be close by, and must be silent and acoustic.⁴² Good lighting, including that of the ambient light sources, screens and monitors, is important. A balance between screen lighting and ambient lighting ensures that the radiologist can observe small details. More studies are required to determine the standard maximum luminance of radiological equipment.^{35,43,44}

To avoid the symptoms associated with CVS, it is recommended to blink continuously, making this exercise a conscious act. A short break should be made by observing distant objects for at least 5 minutes every 2 hours. Computer screens should be placed below the eye level, i.e., the top edge of the monitor should be below the eye

level and further from the eyes than the bottom edge so that there is a downward angle of 14°. The optimum distance from the monitor to the eyes should be at arm's length, or 76–101.5 cm away with a 5-millimeter font size.^{45–47}

Radiologists should perform a simple stretching exercise every hour of work.^{48,49} The chairs utilized by radiologists must be adjustable. Whilst sitting, the radiologist's feet must not dangle, but rather must be supported on the ground forming an angle of 90–105° between the calves and the thighs. The use of arm supports relieves the effort of the muscles of the back. Curved chairs cause upper body ailments, whereas flat chairs cause lower body ailments.^{50,51} An optimal working environment has a temperature of 20–25°C with 40–60% humidity.^{43,52,53} Noise should be minimized as much as possible; however, the maximum noise threshold that should be allowed at the workstation is 58 dB.^{54–56} When using the mouse, the hand and the wrist should be positioned possibly parallel to the desk. A mouse with a slight increase in height in the most anterior part is recommended (Table 1).^{57–60}

Table 1. Occupational diseases and their origin, and recommendations related to the work activities of oral and maxillofacial radiologists

Occupational discomfort	Causes	Ergonomic recommendations
Lower back and upper limb pain	sustained and bad postures, uncomfortable chairs, desks of insufficient or excessive height, limited space	<ul style="list-style-type: none"> – a simple stretching exercise and an active break every hour of work – furniture adjustable to the figure and height of the person – using a chair that can be adjusted to the needs of the person sitting on it and can provide good lumbar support, without causing pressure on the thighs and without the feet hanging; the feet must be supported on the ground at an angle of 90–105°
Carpal tunnel and tenosynovitis	repetitive movements with the hands, sustained and awkward postures while using the mouse and the keyboard, transcription of reports in a bad posture	<ul style="list-style-type: none"> – the mouse and the keyboard should be arranged in such a way so that to minimize the deflection, extension and flexion of the wrist – the mouse should be grasped with the dominant hand and the wrist should be positioned possibly parallel to the desk, a mouse with a slight increase in height in the most anterior part is recommended as well as using gel support pads for the wrists – using a DictaphoneTM
Neck pain	sustained head postures with excessive up or down head movements	<ul style="list-style-type: none"> – placing the computer screen below the eye level so that to ensure is a 14-degree downward gaze
Burnout syndrome	extended working hours, excessive workload, difficult employment relationships, lack of resources to perform duties properly, neglect of facets of life other than work	<ul style="list-style-type: none"> – maintaining a balance between work and family life – coping with dictatorial hierarchies – avoiding severe self-criticism – providing sufficient salary rewarding, having a number of employees according to the work volume, allowing spaces for the members of the work team to integrate, respecting nighttime hours and weekends, not interrupting with calls, avoiding tasks to be done at home, outside the workplace and working hours
Clinical depression	endogenous origin or biological, genetically determined predisposition, enhanced by environmental factors or reactive factors that occur in the face of poor adaptation to stressful environmental factors	<ul style="list-style-type: none"> – occupational medical services regarding mental health focused on medical professionals, including radiologists – controlling any physical illness
CVS	activities that stress near vision and are related to using computers	<ul style="list-style-type: none"> – adequate ambient lighting – full blink, 5-minute breaks every 2 h, yearly eye exams – using anti-glare filters on monitor screens – use software that reduces blue light – temperature of 20–25°C with 40–60% humidity
Suicidal thoughts	depression, anxiety, bipolar disorder, psychoactive substance abuse, alcoholism, loneliness	<ul style="list-style-type: none"> – OH services involving the treatment of depression, bipolar disorder, psychoactive substance abuse, alcoholism, self-medication, and feel-good medication
Metabolic syndrome	high degree of work stress, sedentary lifestyle, unhealthy lifestyle	<ul style="list-style-type: none"> – promoting a healthy lifestyle – physical activity – regular medical exams

CVS – computer vision syndrome; OH – occupational health.

Occupational medical services for medical professionals, including radiologists, should focus on mental health for the early detection of depression, stress, chronic exhaustion, bipolar disorder, psychoactive substance abuse, alcoholism, self-medication to feel good as well as suicide risk factors.

Situations related to lawsuits or possible lawsuits should be managed. Long work hours should be avoided and sufficient sleep is essential. Efforts must be made to maintain a balance between work and family life. A healthy lifestyle must also be promoted by getting physical activity and controlling any physical illness. Work hierarchies that do not tolerate mistakes must be overcome, and also severe self-criticism should be avoided. From an employer's point of view, sufficient salary rewarding should be given according to the context, region, functions, and professional title. Additionally, the number of employees should be in accordance with the work volume. Free-time spaces should be available for the members of the work team to integrate. Nighttime and weekend hours must be respected, and interruptions with calls or tasks outside the workplace and working hours should not be made. In addition, tasks that are needed to be done at home must be avoided. Lastly, it is recommended to spend time outdoors to allow contact with the sun. Exposure to sunlight stimulates the secretion of the hormones serotonin and melatonin, which are related to feelings of well-being and happiness, and sleep.


Conclusions

Oral radiologists are exposed to several risks and occupational diseases inherent and/or related to the work. However, these risks can be avoided by the implementation of the well-documented habits and ergonomic advice that improve a person's overall quality of life.


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Light-activated disinfection in endodontics: A comprehensive review

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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

Light-activated disinfection (LAD) has emerged as a novel approach toward antimicrobial disinfection within the root canal. This approach is based on the concept that porphyrins and photosensitizers (PSs) can be activated by light to produce cytotoxic elements that induce the desired therapeutic effect. Unlike antibiotics, LAD can act on multiple targets within a bacterial cell, including membrane lipids, genomic DNA and various proteins, including enzymes, thus reducing the ability of the organism to acquire resistance.

The aim of this review was to develop an understanding of the potential use of LAD in endodontics and to suggest strategies to maximize the antibacterial effects of LAD.

The electronic searches of the PubMed/MEDLINE, Web of Science, Scopus, and Cochrane databases were complemented by a manual hand search. A total of 303 studies were evaluated for essential parameters, which included the origin, types/variations, methodology, and application of LAD in in vitro and in vivo studies.

It can be concluded that LAD is effective against the vast majority of bacterial pathogens, including antibiotic-resistant Gram-negative and Gram-positive bacteria, along with several yeasts, viruses and protozoan species. The literature tends to suggest that LAD can be used either as a substitute or an adjunct to the conventional antimicrobial treatment regimens that are implemented to battle polymicrobial biofilms.

Keywords: biofilms, disinfection, light-activated disinfection, photosensitizing agents, root canal therapy

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Introduction

There are over 700 microbial species that can be present in the oral cavity, and an individual can have 100–200 species at any given time.¹ Usually, primary root canal infections are polymicrobial in nature and are dominated by anaerobic bacterial species.² The organisms frequently isolated in such cases include Gram-negative anaerobic rods, Gram-positive anaerobic cocci, gram-positive anaerobic and facultative rods, *Lactobacillus*, and *Streptococcus spp.*² Most anaerobes are easy to eliminate during root canal treatment, but facultative bacteria may survive the disinfection procedures.² *Enterococcus faecalis* is the microorganism that has been isolated in most cases of failed root canal treatment, and has therefore been mentioned in the literature as one of the chief causative agents.³ Along with *E. faecalis*, *Staphylococcus*, *Enterococcus*, *Enterobacter*, *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Sphingomonas*, *Candida*, and *Actinomyces spp.* have also been isolated from root-filled teeth with post-treatment disease.^{4–9} Antibacterial agents are widely used in the treatment of bacterial infections, but the emergence of bacterial pathogens resistant to the commonly used chemotherapeutics has led to a search for alternative drugs and/or therapies to overcome the development of resistant species.

Sodium hypochlorite (NaOCl) is the gold standard for endodontic disinfectants,¹⁰ as it has the ability to dissolve tissue and provide broad-spectrum antimicrobial effects,¹¹ making it the solution of choice for the treatment of pulp necrosis and infection.¹² However, this disinfectant has several undesirable drawbacks, such as the risk of tissue damage, allergic potential, and unpleasant smell and taste. Although other irrigants, such as chlorhexidine, are more compatible than NaOCl, they lack the tissue dissolving ability; thus, their activity is greatly reduced when exposed to organic matter.¹³ Several other irrigants have been used for endodontic disinfection, but have been found to be inferior to or equally effective as (with regard to bactericidal properties) NaOCl.¹⁴

These drawbacks have forced a major research effort to find alternative antimicrobial approaches aimed at killing microorganisms without causing resistance. The concept of light-based disinfection as a means of eliminating the bacterial microflora from within the root canal was described by Foote.¹⁵ Light-activated disinfection (LAD) has emerged as a novel approach toward antimicrobial disinfection within the root canal.¹⁶ It is based on the concept that porphyrins and photosensitizers (PSs) can be activated by light to produce cytotoxic elements that induce the desired therapeutic effect.¹⁶ Light-activated disinfection can act on multiple targets within a bacterial organism. These target sites include the lipid membrane, genomic DNA and various proteins, including enzymes. This, in turn, reduces the ability of the organism to acquire resistance against LAD.¹⁶

The aim of this review was to develop an understanding of the potential use of LAD in endodontics and to suggest strategies to maximize the antimicrobial effects of this technique.

Methodology

The electronic searches of the PubMed/MEDLINE, Web of Science, Scopus, and Cochrane databases were carried out. A total of 303 studies were evaluated for essential parameters, which included the origin, types/variations, methodology, and application of LAD, along with the potential risk factors reported in in vitro and in vivo studies. The searches were carried out using the combinations of the following keywords: “microbial infections”; “porphyrins”; “photosensitization”; “activated oxygen”; “bacterial infections/therapy”; “phototherapy”; “diode laser”; “blue light”; and “wavelength 450–670 nm.” After the initial screening, a total of 80 articles were selected. The electronic searches were complemented by a manual search of various textbooks and articles. A total of 7 articles were identified as a result of the manual search. In total, 87 articles were considered relevant and used for this project (Fig. 1).

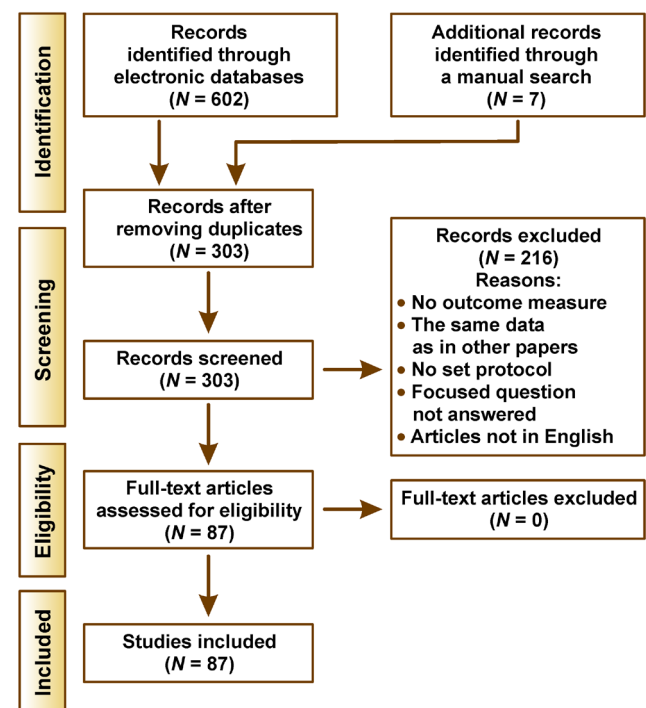


Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram showing the literature search and the selection criteria. According to: Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA statement. *PLoS Med.* 2009;6(7):e1000097. doi:10.1371/journal.pmed.1000097. For further information, visit <http://www.prisma-statement.org/>.

Light-activated disinfection in endodontics

Light-activated disinfection starts when the porphyrins or PSs are exposed to a specific wavelength of light, within the target tissue, leading to the production of singlet oxygen ($^1\text{O}_2$), being the main reactive oxygen species (ROS) (Fig. 2).

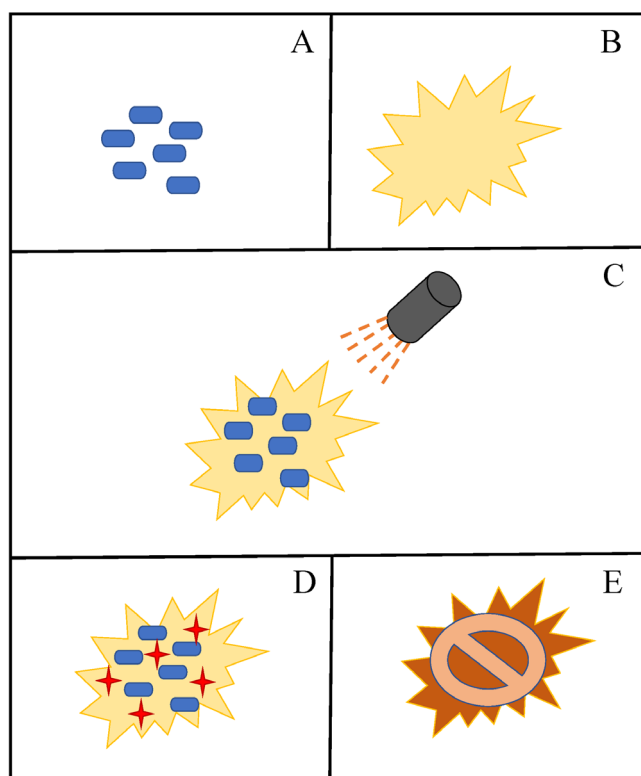


Fig. 2. Mechanism of action of light-activated disinfection (LAD) on a bacterial cell

A – photosensitizer (PS) molecules; B – bacterial cell; C – PS entering the bacterial cell, followed by activation with the use of light of a specific wavelength; D – production of reactive oxygen species (ROS); E – cell death.

Effect on bacterial biofilms

Most of the laboratory and clinical investigations using the LAD technique within the root canal use a PS rather than bacterial porphyrins. Photosensitizers are chemical derivatives of the naturally occurring porphyrins within the specific species. The effective elimination of both *Streptococcus mutans* and *E. faecalis* has been reported with a combination of LAD and either methylene blue (MB) or toluidine blue O (TBO), with a killing efficacy of 97–99.9% for planktonic bacterial loads of up to 10 million organisms at an exposure time of 120 s.¹⁷ Relatively similar results have been reported for the elimination of *Staphylococcus intermedius*, with complete kills for loads of up to 1,000 million organisms within the root canal, using TBO as a dye and a helium-neon (He-Ne) laser

of an output power of 35 mW, when exposed for 150 s.¹⁸ When *E. faecalis* is used as the infecting organism, there is a reported 77.5% killing rate with a combination of MB and a diode laser at a fluence level of 60 J/cm², 99.9% with TBO and a laser of an output power of 50 mW at an energy level of 6.4 J,¹⁹ and 90% killing ex vivo and 99.99% killing in vitro while using a combination of TBO and a diode laser of an output power of 100 mW at an energy level of 15 J.²⁰ According to George and Kishen, 99.99% elimination of *E. faecalis* biofilms could be achieved by using MB and a 30 mW diode laser set at 36 J.²¹ By applying a dual-stage approach (a modified PS formulation and an irradiation medium), they managed to achieve disinfection without canal enlargement. This procedure was termed “advanced non-invasive light-activated disinfection” or ANILAD.²¹ The use of LAD has also been shown to be effective against *Prevotella intermedia*, *Peptostreptococcus micros*, *Fusobacterium nucleatum*, *Porphyromonas spp.*, and *Actinomyces spp.*²¹

Studies have also shown the effectiveness of LAD in eradicating mixed biofilm infections. Fimple et al. suggested that, when combined with MB, diode lasers could cause a 73–80% reduction in multi-species bacterial biofilm loads.²² An in vitro study by Soukos et al. on planktonic biofilms showed that all microorganisms were eliminated following MB-mediated LAD, except *E. faecalis*, which showed only a 47% reduction.²³ However, the authors did report a 97% reduction of *E. faecalis* on *E. faecalis*-based biofilms afterward. The authors suggested that the variation was due to a difference in susceptibility toward much higher energy fluence for LAD that was being used.²³ In another study, Williams et al. compared the sensitivity of planktonic microorganisms against the biofilms grown in root canals and Perspex[®] simulated canals.²⁴ The specimens were exposed to a combination of LAD with TBO. The results indicated that LAD was less effective in root canals than in the suspension form. The study did not run a comparison for single species within the planktonic and biofilm mode of growth.²⁴ It should also be noted that clinically, most acute exacerbations during endodontic treatment involve the *Porphyromonas* bacterial species,²⁵ in particular *P. endodontalis*, an anaerobe that is highly susceptible to $^1\text{O}_2$.

Use of LAD with the existing irrigation methods

Light-activated disinfection should be used in conjunction with the existing measures of irrigation, such as the use of NaOCl. In a study by Bonsor et al., 14 patients were evaluated to assess the efficacy of TBO and a diode laser in combination with the conventional root canal treatment.²⁶ The results showed a 96.7% bacterial reduction.²⁶ Another study by the same authors included 64 patients and used a chelating agent (ethylenediaminetetraacetic acid – EDTA) before the use of LAD.²⁷ The results also showed

a significant bacterial reduction.²⁷ Garcez et al. conducted a study on 20 patients and the initial exposure to LAD resulted in a 98.5% bacterial reduction.²⁸ The treatment was followed up with a calcium hydroxide (Ca(OH)₂) dressing for 1 week before another round of LAD exposure, resulting in a 99.9% bacterial killing rate. The authors suggested that the use of LAD before and after Ca(OH)₂ was more effective than the initial dosage.²⁸

Previous studies have shown that typical LAD parameters for the effective killing of microbes are on the order of 15 J/cm² delivered using a visible red diode laser with an output power of up to 100 mW over 60–120 s.^{17,29–32} Lee et al. provided certain guidelines for the use of LAD in a clinical environment.³³ They suggest that PS should be placed in direct contact with the infected site for a short period, allowing the microorganism to absorb as much of the reactive agent as possible. This would increase sensitivity to light. Also, the dye must be agitated within the canal to eliminate air bubbles that could impede contact with the bacteria.³³ The photosensitizer must also be applied into a root canal space that is free of blood and saliva, as these can potentially impair the efficacy of photosensitization.³⁴ In addition, to achieve maximal effects of the laser energy, it should be delivered through a diffuser tip, thus providing a narrow cylindrical pattern of light emission.³³ The emission pattern also follows the shape of the root canal.³³ Diffuser tips reduce power density, which, in turn, reduces the risk of optical injury.³³

A study measuring a temperature rise in the root canal during LAD reported a value of 0.16 ± 0.08°C.³⁵ This is lower than the reported 7°C safety level for periodontal injury.³⁵ Another study measuring thermal effects during LAD suggested that a change in temperature was less than 0.5°C.¹⁷ This change was not said to be clinically significant, since the critical threshold levels for irreversible pulpitis is 11 times higher, at 5.5°C.³⁵ This seems to suggest that, with regard to the concerns about the adverse effects due to a rise in temperature in the root canal, using LAD for endodontic disinfection can be considered harmless to the surrounding periodontal tissues.³⁵

Strategies to maximize bacterial killing by LAD

Pre-treatment of cells with membrane permeabilizing agents

Nitzan et al. and other researchers suggested that the application of polycationic polypeptide polymyxin B nonapeptide (PMBN) prior to LAD exposure increased the permeability of the outer cell membrane of various Gram-negative bacteria.^{36–39} This treatment allows a greater penetration of the photosensitizing agent in situations where the supply of ROS is low. The application of PMBN does

not cause the release of lipopolysaccharides (LPSs) from the cell; rather it causes the outer membrane to expand, resulting in an increased penetration of PS. A study by Walther et al. concluded that following pre-treatment with PMBN, Gram-negative *Yersinia pseudotuberculosis* and *Escherichia coli* had an increased susceptibility to a combination of LAD exposure and protochlorophyllide.⁴⁰ In a similar approach, Yonei and Todo showed that the lethal effects of EDTA increased when the *E. coli* samples were exposed to LAD beforehand.⁴¹ This may be due to the presence of chlorpromazine in EDTA.⁴¹ Also, EDTA can stimulate the release of LPSs in *E. coli* treated with calcium chloride (CaCl₂) when LAD is used with either rose bengal or hematoporphyrin/zinc phthalocyanines.⁴²

Modification of the photosensitizer

In a study by Bezman et al., the authors were able to covalently bind rose bengal to polystyrene beads mixed in a bacterial suspension.⁴³ The authors concluded that this approach enabled PS to form ROS that could penetrate more easily and efficiently through the outer cell membrane.⁴³ This is similar to the work by Friedberg et al., who were able to bind PSs to monoclonal antibodies.⁴⁴ These antibodies could attach themselves to the surface antigens present on *Pseudomonas aeruginosa*, which resulted in the specific killing of the target bacteria.⁴⁴ Wilson applied phenothiazinium TBO and LAD on a variety of both Gram-positive and Gram-negative bacteria, achieving significant eradication rates.⁴⁵ Similar results were reported by Usacheva et al.⁴⁶ and George and Kishen,⁴⁷ where the authors used phenothiazinium dyes to inactivate Gram-positive and Gram-negative bacteria.

Soukos et al. suggested that it might be possible to covalently bond a photosensitizing agent to a poly-L-lysine chain.⁴⁸ This delivery vehicle could effectively inactivate a variety of bacterial species. The authors demonstrated that by conjugating chlorine e6 and a poly-L-lysine chain made up of 20 lysine molecules, a killing rate of over 99% for *Actinomyces viscosus* (Gram-positive) and *Porphyromonas gingivalis* (Gram-negative) could be achieved.⁴⁸ Similar results were reported by Rovaldi et al., where the authors used a construct of 1 chlorine e6 and a 5-amino acid lysine chain,⁴⁹ and by Hamblin et al., where the authors described the effects of a poly-L-lysine-chlorine e6 conjugate of 37 lysines bound with 1 chlorine e6 molecule against both Gram-positive and Gram-negative species, achieving a significantly high killing rate.⁵⁰

5-ALA porphyrin stimulation

Kennedy and Pottier reported the possibility of increasing the amount of porphyrins present in bacterial species that do not have the natural tendency to produce endogenous porphyrins.⁵¹ This was achieved by adding exogenous 5-aminolevulinic acid (5-ALA).⁵¹ The inactivation

of *E. coli* after incubation in 5-ALA and exposure to white light was shown by Gábor et al.⁵² However, *Enterococcus hirae* could not be eradicated with this approach.⁵²

Alteration of the photosensitizer

Studies have also been conducted to improve the efficacy of the LAD process.^{53,54} George and Kishen mixed MB with water, 70% glycerol, and 70% polyethylene glycol (PEG) in a proportion of glycerol:ethanol:water (MIX) of 30:20:50.⁴⁷ Their results indicated that the molecules of MB aggregated at a greater rate in $^1\text{O}_2$ water as compared to the other aqueous media. The combination of MB with the MIX formulation produced greater bactericidal activity. This is believed to be due to a combined effect of an increased penetration of MIX within the dentinal tubules, the enhanced photooxidation of the model substrate and an increased rate of production of $^1\text{O}_2$.⁴⁷ A follow-up study suggested that, when compared with water, MIX resulted in an increased level of damage to the cell wall and chromosomal DNA.⁵⁵ The same authors also indicated that the alteration of the formula by the addition of an oxidizing agent and O_2 resulted in a more efficient disinfection of the endodontic biofilm.⁵⁵ The altered emulsion was composed of perfluoro(decahydronaphthalene) (oxygen carrier) and hydrogen peroxide (oxidizer) mixed with the detergent Triton®-X100.⁵⁵

Efflux pump inhibitors

Prokaryotic and eukaryotic families have membrane proteins called “efflux pumps”, which aid in the removal of amphiphilic molecules from the cell.⁵⁶ These molecules combine hydrophobic properties, which facilitate cell penetration, and hydrophilic properties, which allow the distribution of compounds to tissues within the body. Many of the drugs available are amphiphilic; hence, efflux pumps tend to remove these molecules effectively from the cell.⁵⁷ Efflux is suggested to be a significant contributor toward bacterial survival (Fig. 3).⁵⁸ Inhibiting this process could potentially restore the ability of antimicrobials to decrease bacterial resistance. Kvist et al. indicated that efflux pumps were generally highly active within biofilms, therefore making them good targets to help prevent biofilm formation.⁵⁹

In addition, the amphiphilic cations have an inhibitory effect on efflux pumps; therefore, phenothiazinium dyes can act as substrates for the microbial efflux pumps, as they are structurally similar to the amphiphilic cations.⁶⁰ Indeed, it has been demonstrated that the inhibition of efflux pumps, along with phenothiazinium dyes, increases the efficacy of LAD.⁶¹ However, there are no current clinical applications using these efflux pump inhibitors. This could be due to the increased levels of toxicity observed with these compounds, which has been reported in animal studies.⁶²

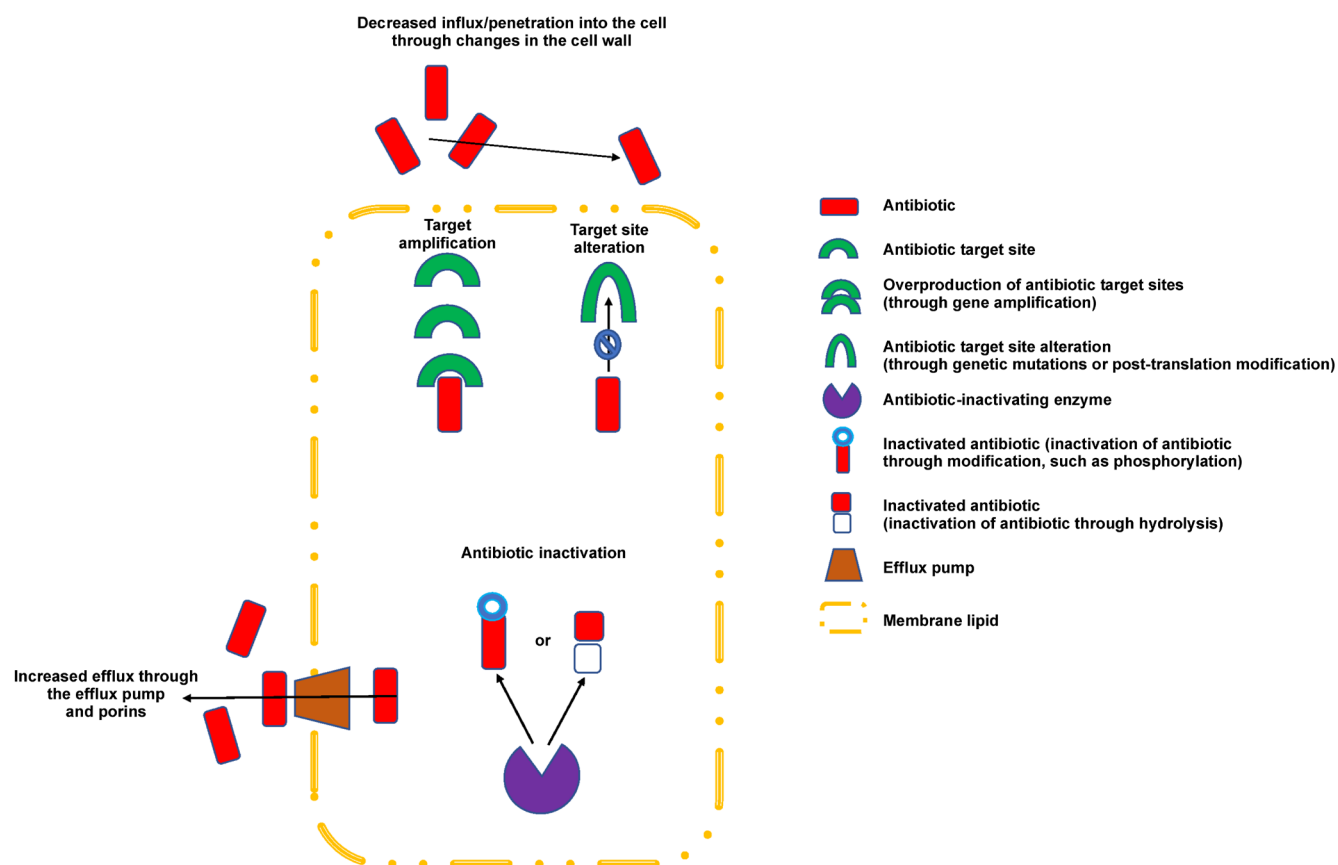


Fig. 3. Schematic diagram highlighting the efflux pump antibiotic resistance mechanisms utilized by bacteria

Factors limiting the efficacy of LAD

The light source is a limiting factor for the penetrative ability of LAD. Light can be either coherent (lasers) or non-coherent (lamps).⁶³ The type of light required is dependent on the location, dosage, and PSs or porphyrins being used. Lasers provide powerful monochromatic light that reduces the delivery time of LAD. As lasers are monochromatic, the wavelength plays a crucial role in the LAD process, as it should match the absorption bands of PSs or porphyrins.⁶³ This often means that a combination of different lasers may be required to achieve the desired result. The laser systems used in various LAD studies include argon (Ar)/dye lasers, He-Ne lasers, potassium titanyl phosphate/neodymium-doped yttrium aluminum garnet (KTP/Nd:YAG)/dye lasers, and diode lasers (Table 1 and Table 2). Presently, lasers are the source of choice when used to irradiate areas accessible only with the aid of optical fibers. The beam quality and the output power are characteristics that make lasers highly effective when coupled with optical fiber cores smaller than 500 µm in diameter.⁶³

In comparison to lasers, lamps cannot be used in combination with small optical fibers, as their poor beam quality, large beam size and low power densities make them inefficient for use in smaller areas. Lamps, however, can be used directly or coupled with a liquid light guide of 5–10 mm in diameter. Both lamps and lasers have

been used in LAD and neither is shown to be better than the other based on their application. Although LAD has been traditionally performed using lasers, the availability of broad-band sources (lamps) is challenging the use of lasers.⁶³ The scattering of light in tissues has a pronounced effect on light intensity and directionality. Along with refraction, it causes a widening of the light beam, thus lowering the fluence rate (energy per unit area) of the light, which results in a change of the direction of the light. Williams et al. used LAD combined with TBO on *S. intermedius* with a diode laser at 633 nm and an output power of 80 mW.²⁴ The organism was irradiated for 30 s, 60 s and 90 s at energy doses of 2.4 J, 4.8 J and 7.2 J, respectively. The authors concluded that the effectiveness of LAD increased with an increase in the dosage of energy.²⁴ However, care must be taken, as the extensive use of light in this range could be harmful for the host cells.

Conclusions

Light-based disinfection is a promising novel approach for root canal disinfection, as studies have indicated its effectiveness against a vast majority of pathogens, including Gram-negative and Gram-positive bacteria. Light-activated disinfection targets multiple sites within the bacterial cell, therefore limiting the ability of the pathogens to acquire resistance. Moreover, it has been suggested that

Table 1. Light and dye parameters applied in some in vitro studies on the use of LAD in endodontics

Study (year)	Model	Light and irradiation parameters	Photosensitizer (formula)	Results
Seal et al. (2002) ¹⁸	2-day biofilms of <i>Staphylococcus intermedius</i>	He-Ne gas laser at 632.8 nm P = 35 mW E = 2.1, 3.2, 4.2, 10.5, or 21 J	TBO (C ₁₅ H ₁₆ N ₃ S ⁺)	maximum of 5 log ₁₀ reduction in CFU/mL at 21 J
Soukos et al. (2006) ²³	3-day biofilms of <i>Enterococcus faecalis</i>	diode laser at 665 nm PD = 740 mW/cm ² F = 222 J/cm ²	MB (C ₁₆ H ₁₈ ClN ₃ S)	97% reduction in bacterial viability
George and Kishen (2007) ²¹	4-day biofilms of <i>E. faecalis</i> and <i>Aggregatibacter actinomycetemcomitans</i>	diode laser at 664 nm P = 30 mW E = 36 J	MB (C ₁₆ H ₁₈ ClN ₃ S)	≥5 log ₁₀ reduction in CFU/mL
Fonseca et al. (2008) ¹⁹	2-day biofilms of <i>E. faecalis</i>	Ga-Al-As diode laser P = 50 mW E = 6.4 J	TBO (C ₁₅ H ₁₆ N ₃ S ⁺)	≈99.9% reduction in bacterial viability
Fimple et al. (2008) ²²	3-day multi-species biofilm	diode laser at 665 nm PD = 100 mW/cm ² F = 30 J/cm ²	MB (C ₁₆ H ₁₈ ClN ₃ S)	≈73–80% reduction in bacterial viability
Meire et al. (2009) ²⁰	2-day biofilms of <i>E. faecalis</i>	diode laser at 635 nm P = 100 mW E = 15 J	TBO (C ₁₅ H ₁₆ N ₃ S ⁺)	≈1.5 log ₁₀ reduction in CFU/mL
Aydin et al. (2020) ⁶⁵	28-day incubation of <i>E. faecalis</i>	diode laser at 628 nm P – not mentioned	TBO (C ₁₅ H ₁₆ N ₃ S ⁺)	97.8911% reduction in <i>E. faecalis</i> bacterial load
Yoshii et al. (2021) ⁶⁴	2-day biofilms of <i>Lactobacillus acidophilus</i>	laser at 650 and 940 nm P = 9 mW and 600 mW E – not mentioned	AR (C ₂₇ H ₂₉ N ₂ NaO ₇ S ₂) and BB (C ₃₇ H ₃₄ N ₂ Na ₂ O ₉ S ₃)	650-nm laser combined with the BB solution was most effective in sterilizing the dentin plates infected with <i>L. acidophilus</i>

He-Ne – helium-neon; P – output power; E – energy; PD – power density; F – fluence; Ga-Al-As – gallium-aluminum-arsenide; TBO – toluidine blue O; MB – methylene blue; AR – acid red; BB – brilliant blue; CFU – colony-forming unit.


Table 2. Light and dye parameters applied in some in vivo studies on the use of LAD in endodontics


Study (year)	Model	Light and irradiation parameters	Photosensitizer (formula)	Results
Bonsor et al. (2006) ²⁷	64 canals in patients with symptoms of irreversible pulpitis/apical periodontitis	diode laser at 633 nm P = 100 mW E = 12 J	TBO (C ₁₅ H ₁₆ N ₃ S ⁺)	>90% reduction in bacterial viability with the use of a chelating agent along with LAD
Garcez et al. (2008) ²⁸	20 single-rooted canals in patients with symptoms of necrotic pulp and apical periodontitis	diode laser at 660 nm P = 40 mW E = 9.6 J	PEI (C ₂ H ₅ N) _n and chlorin e6 (C ₃₄ H ₃₆ N ₄ O ₆) conjugate	≈99.9% reduction in bacterial viability following 2 successive combinations of the conventional endodontic therapy and LAD
Zorita-García et al. (2019) ⁶⁶	42 single-rooted teeth in 33 patients with apical periodontitis	diode laser at 630 nm PD = 2,000 mW/cm ²	TBO (C ₁₅ H ₁₆ N ₃ S ⁺)	90.3% reduction in <i>E. faecalis</i> bacterial load


PEI – polyethylenimine

LAD can be used either as a substitute or an adjunct to the conventional antimicrobial treatment regimens used for battling polymicrobial biofilms. However, it is the authors' suggestion that further studies be conducted, e.g., incorporating nanocarrier systems for PS to evaluate its effect on various biofilms that are persistent in root canal infections.

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