Dental and Medical Problems

QUARTERLY ISSN 1644-387X (PRINT) ISSN 2300-9020 (ONLINE) www.dmp.umed.wroc.pl

2022, Vol. 59, No. 1 (January–March)

Ministry of Science and Higher Education – 70 pts
Index Copernicus (ICV) – 128.41

Wroclaw Medical University

Dental and Medical Problems

ISSN 1644-387X (PRINT) ISSN 2300-9020 (ONLINE) www.dmp.umed.wroc.pl

QUARTERLY
2022, Vol. 59, No. 1
(January–March)

„Dental and Medical Problems" is an international, peer-reviewed, open access journal covering all aspects of oral sciences and related fields of general medicine, published quarterly by Wroclaw Medical University.

Editorial Office
Marcinkowskiego 2–6
50-368 Wrocław, Poland
Tel.: +48 71 784 12 05
E-mail: dental@umed.wroc.pl

Publisher
Wroclaw Medical University
Wybrzeże L. Pasteura 1
50-367 Wrocław, Poland

Online edition is the original version of the journal

Manuscript editing
Joanna Gudarowska
Jolanta Krzyżak
Marek Misiak

Editorial Policy

During the review process, the Editorial Board conforms to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication" approved by the International Committee of Medical Journal Editors (http://www.icmje.org/). Journal accepts only original papers and all kinds of reviews (narrative review, systematic review, systematic review and meta-analysis, etc.). Experimental studies must include a statement that the experimental protocol and informed consent procedure were in compliance with the Helsinki Convention and were approved by an ethics committee.

Indexed in: PubMed, Web of Science, Scopus, ICI Journals Master List, DOAJ, GFMER, CiteFactor, WorldCat, Embase, Polska Bibliografia Naukowa, EBSCO, Crossref, CLOCKSS

Typographic design: Monika Kolęda, Piotr Gil
Cover: Monika Kolęda
DTP: Adam Barg
Printing and binding: Soft Vision sp. z o.o.

Dental
and Medical Problems

QUARTERLY 2022, Vol. 59, No. 1 (January–March)

ISSN 1644-387X (PRINT)
ISSN 2300-9020 (ONLINE)
www.dmp.umed.wroc.pl

Contents

Original papers

Reviews

Preventive measures for COVID-19 among dental students and dentists during the mandatory social isolation in Latin America and the Caribbean in 2020

Brenda Melissa Flores-Quispe[1,A–F], Renato Alejandro Ruiz-Reyes[1,A–F], Roberto Antonio León-Manco[1,A–F], Andrés Agudelo-Suárez[2,A–F]

[1] Faculty of Dentistry, Cayetano Heredia Peruvian University, Lima, Peru
[2] Faculty of Dentistry, University of Antioquia, Medellín, Colombia

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):5–11

Address for correspondence
Brenda Melissa Flores-Quispe
E-mail: brenda.flores.q@upch.pe

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on June 19, 2021
Reviewed on August 15, 2021
Accepted on September 8, 2021

Published online on February 1, 2022

Abstract

Background. Dentistry is one of the professions that are most exposed to the contagion with coronavirus disease 2019 (COVID-19). However, the prevalence and positivity rates of COVID-19 are low in dentists, indicating that the current measures of infection control may be sufficient to prevent infection in dental settings.

Objectives. The aim of the study was to determine whether the preventive measures for COVID-19 during the mandatory social isolation were followed by dental students and dentists in Latin America and the Caribbean in 2020.

Material and methods. A cross-sectional study was conducted using anonymous virtual surveys in a convenience sample of 2,036 dental students and dentists from 21 Latin American and Caribbean countries. The variables were the preventive measures for COVID-19 and the sociodemographic characteristics. Descriptive and bivariate analyses were performed.

Results. The final sample included 2,036 dental students and dentists. The self-perceived level of knowledge about COVID-19 was found to be associated with age, sex, body mass index (BMI), the type of academic training, having a specialty in the case of professionals, the place of origin, and having met someone with COVID-19 ($p < 0.05$). The self-perceived level of concern regarding COVID-19 was associated with sex, BMI and having met someone with COVID-19 ($p < 0.05$). The number of days in the mandatory social isolation was associated with age, the type of academic training, having a specialty, the place of origin, and having met someone with COVID-19 ($p < 0.05$). The confinement level was associated with age, sex, BMI, the type of academic training, and having met someone with COVID-19 ($p < 0.05$). Following the preventive measures for COVID-19 was associated with age, the type of academic training, having a specialty, the place of origin, and having met someone with COVID-19 ($p < 0.05$). The use of face masks, hand washing and social distancing were associated with age, BMI, the type of academic training, the place of origin, and having met someone with COVID-19 ($p < 0.05$).

Conclusions. Dental students and dentists followed the preventive measures for COVID-19 during the mandatory social isolation period in Latin America and the Caribbean in 2020.

Keywords: oral health, prevention and control, COVID-19, coronavirus infections

Cite as
Flores-Quispe BM, Ruiz-Reyes RA, León-Manco RA, Agudelo-Suárez A. Preventive measures for COVID-19 among dental students and dentists during the mandatory social isolation in Latin America and the Caribbean in 2020. *Dent Med Probl.* 2022;59(1):5–11. doi:10.17219/dmp/142033

DOI
10.17219/dmp/142033

Introduction

At the end of December 2019, the Chinese Ministry of Health notified the World Health Organization (WHO) about numerous cases of pneumonia of unknown etiology in Wuhan, China.[1,2] On January 7, 2020, the causative agent of this enigmatic pneumonia was identified as a novel coronavirus (2019-nCoV) by analyzing a throat swab taken from one of these patients.[2,3] The Coronavirus Study Group named this pathogen "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)",[2,4] and the WHO named the disease caused by the virus "coronavirus disease 2019 (COVID-19)".[2,5] The WHO declared the SARS-CoV-2 outbreak a public health emergency of international concern (PHEIC) on January 30, 2020.[2,5]

There continues to be uncertainty regarding the propagation mechanism; 2019-nCoV uses the same receptor as SARS-CoV – angiotensin-converting enzyme 2 (ACE2). The principal mechanism of infection is via aerosol transmission by person-to-person contact, which occurs by respiratory droplets, but it can also be transmitted by the contaminated hands or surfaces. Transmission is possible for approx. 8 days since the occurrence of symptoms.[6] Patients may still have a positive pharyngeal smear result for several weeks after symptoms resolve.[6] The most common symptoms are cough, the shortness of breath, chest pain or pressure, a high fever (over 38°C), a sore throat, diarrhea, headache, muscle or joint pain, fatigue, and the loss of sense of taste and smell.[7] The pandemic unleashed by COVID-19 has changed people's lifestyles due to the ease of transmission and its high mortality rate in geriatric patients and patients with pre-existing diseases. The main measures to combat the virus are vaccination, standard preventive measures, such as the use of masks and social distancing, and timely treatment for those with symptoms. Countries have adopted different forms of mandatory social isolation, making it difficult for people to enjoy normal daily life and free movement while impacting education and the economy.[8] The pandemic has also had a major psychological impact by adding to the prevalence of anxiety and depression in the general population, which is increased in people with chronic diseases and, especially, in health care workers, who are constantly under stressful conditions.[9]

Dentistry is one of the professions with the greatest exposure to COVID-19.[10] This is due to the fact that the surfaces of both dental units and the contaminated materials act as major sources of infection during daily clinical practice for both the patient and the professional, especially with the use of rotary equipment.[11,12] However, the American Dental Association (ADA) reported that the prevalence and positivity rates of COVID-19 were low in dentists, indicating that the present measures of infection control may be sufficient to minimize risk in dental environment.[13] Among the measures adopted for dental practices during the pandemic is the use of personal protective equipment (PPE), which consists of face shields, glasses, masks, gloves, one-piece uniforms, caps, and disposable boots.[14] At the beginning of the pandemic, treatment for health emergencies only was ordered, following the established protection protocols.[15]

In Latin America and the Caribbean, the first country to have case reports was Brazil on February 25, 2020.[16] In a matter of weeks, countries across the region closed their borders and imposed restrictions. The response of many Latin America and Caribbean countries has not been entirely favorable, observing cases in which there are not enough ventilators to meet patient demand. As of January 13, 2021, a total of 16,724,800 cases of COVID-19 had been registered in Latin America and the Caribbean. Among the countries most affected are Brazil, Colombia, Argentina, Mexico, Peru, and Chile.[17] For this reason, various investigations have been carried out to analyze the impact of COVID-19 on dentists in this region.[18]

The aim of the study was to discuss the preventive measures for COVID-19 during the mandatory social isolation period among dental students and dentists in Latin America and the Caribbean in 2020.

Material and methods

Design, data collection and setting

A cross-sectional study was conducted based on the anonymous virtual surveys targeted at dental students and dentists aged 18–71 from 21 countries in Latin America and the Caribbean (Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Dominica, Ecuador, El Salvador, Grenada, Guatemala, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Puerto Rico, Dominican Republic, Uruguay, and Venezuela). The information was organized by the Faculty of Dentistry of the University of Antioquia, Medellín, Colombia, in an Excel database with the records of another survey-based original study.[18] The survey was designed on the Google Forms platform. Due to time restrictions, a pilot study was carried out with a sample of only 30 participants to evaluate internal consistency and completion time; this is a limitation of the study. The survey was available online and distributed digitally between March and August 2020. It collected the sociodemographic data and incorporated questions about the COVID-19 pandemic. It is important to mention that, for this study in particular, the records of all dental students and dentists from Latin America and the Caribbean were evaluated. Records that did not include all the study variables were discarded, so a final sample of 2,036 records was obtained.

Variables

The self-perceived level of knowledge about COVID-19 and the self-perceived level of concern regarding COVID-19 were measured using a Likert 1–10 scale,

where 1 is the lowest rating and 10 is the highest. The number of days in the mandatory social isolation was measured in days, and the confinement level was categorized into "I have not gone out any day", "I have gone out very little", "I have gone out frequently", and "I have gone out every day". Whether the participants followed the preventive measures for COVID-19 was categorized into 'never', 'rarely', 'sometimes', 'usually', and 'always'. The use of face masks, hand washing and social distancing were evaluated with the yes/no options.

The sociodemographic characteristics were age, sex and the body mass index (BMI). The survey also determined the type of academic training, specialty, the location of origin (grouped in Mexico, Central America, the Caribbean together, and South America), whether the participants had met someone with COVID-19, and the COVID-19 information media.

Statistical analysis

A descriptive analysis of the variables was carried out. Subsequently, the use of non-parametric tests was determined using the Kolmogorov–Smirnov test. The Mann–Whitney *U* test was applied for dichotomous variables and the Kruskal–Wallis test was used for polytomous variables in the case of quantitative variables. For the qualitative variables, the χ^2 test for trend was used for ordinal scales. The study had a confidence level of 95% and $p < 0.05$ was considered to be statistically significant for all tests. The IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA) was used for the statistical analysis.

Results

The most frequently selected age range was 25–34 years at 36.79% (n = 749). There was a female predominance at 70.38% (n = 1,433). In relation to BMI, 62.41% (n = 1242) of the participants were at their normal weight. The majority were dentists at 64.44% (n = 1,312), of which 69.59% (n = 913) had a specialty. South America accounted for 91.94% (n = 1,872) of the respondents. In terms of contact, 48.43% (n = 986) had met someone with COVID-19, and 97.00% (n = 1,975) received their COVID-19 information through virtual media. According to the self-perceived level of knowledge about COVID-19, the median (*Me*) was 8.00 (quartile 1 (*Q1*)–quartile 3 (*Q3*): 7.00–8.00). The self-perceived level of concern regarding COVID-19 presented *Me* = 8.00 (7.00–10.00). The median number of days in the mandatory social isolation was 60.00 (55.00–72.00) and 79.27% (n = 1,614) reported having gone out very little outside their confinement. As for following the preventive measures for COVID-19, 69.74% (n = 1,420) reported that they always followed them; 91.16% (n = 1,856) used face masks and employed hand washing and social distancing (Table 1).

Table 1. Characteristics of dental students and dentists during the mandatory social isolation (N = 2,036)

Variable		Value
Age [years]	*M* ±*SD*	32.69 ±11.76
	18–24	606 (29.76)
	25–34	749 (36.79)
	≥35	681 (33.45)
Sex	male	603 (29.62)
	female	1,433 (70.38)
BMI[#] (N = 1,990) [kg/m^2]	underweight	83 (4.17)
	normal	1,242 (62.41)
	overweight	531 (26.68)
	obesity	134 (6.73)
Type of academic training	dental student	724 (35.56)
	dentist	1,312 (64.44)
Specialty[#] (N = 1,312)	yes	913 (69.59)
	no	399 (30.41)
Place of origin	Mexico, Central America and the Caribbean	164 (8.06)
	South America	1,872 (91.94)
Met someone with COVID-19	yes	986 (48.43)
	no	1,050 (51.57)
COVID-19 information media	traditional	61 (3.00)
	virtual	1,975 (97.00)
Self-perceived level of knowledge about COVID-19	*Me* (*Q1*–*Q3*)	8.00 (7.00–8.00)
Self-perceived level of concern regarding COVID-19	*Me* (*Q1*–*Q3*)	8.00 (7.00–10.00)
Number of days in the mandatory social isolation	*Me* (*Q1*–*Q3*)	60.00 (55.00–72.00)
Confinement level	I have not gone out any day	214 (10.51)
	I have gone out very little	1,614 (79.27)
	I have gone out frequently	117 (5.75)
	I have gone out every day	91 (4.47)
Following the preventive measures for COVID-19	never	2 (0.10)
	rarely	6 (0.29)
	sometimes	47 (2.31)
	usually	561 (27.55)
	always	1,420 (69.74)
Use of face masks, hand washing and social distancing	yes	1,856 (91.16)
	no	180 (8.84)

Unless marked otherwise, data presented as number (percentage) (n (%)).
BMI – body mass index; *M* – mean; *SD* – standard deviation; *Me* – median; *Q1* – quartile 1; *Q3* – quartile 3; COVID-19 – coronavirus disease 2019;
[#] responses with missing values.

The self-perceived level of knowledge about COVID-19 was associated with age ($p < 0.001$), sex ($p = 0.024$), BMI ($p = 0.038$), the type of academic training ($p < 0.001$), having a specialty in the case of professionals ($p = 0.035$), the place of origin ($p < 0.001$), and having met someone with COVID-19 ($p < 0.001$). The self-perceived level of concern regarding COVID-19 was associated with sex ($p < 0.001$), BMI ($p = 0.039$) and having met someone with COVID-19 ($p < 0.001$) (Table 2).

Discussion

COVID-19 is spread mainly person-to-person, most often by inhaling particles that are spread by an infected person. Therefore, vaccination and the monitoring of preventive measures are essential, especially in highly exposed professionals like dentists.[19,20] The knowledge about COVID-19 was associated with all variables except the COVID-19 information media. Kinariwala et al. found

Table 2. Knowledge and concern regarding coronavirus disease 2019 (COVID-19) of dental students and dentists during the mandatory social isolation ($N = 2,036$)

Variable		Self-perceived level of knowledge about COVID-19	*p*-value	Self-perceived level of concern regarding COVID-19	*p*-value
Age [years]	18–24	7.00 (6.00–8.00)	<0.001‡*	9.00 (7.00–10.00)	0.195‡
	25–34	7.00 (7.00–8.00)		8.00 (7.00–10.00)	
	≥35	8.00 (7.00–9.00)		8.00 (7.00–10.00)	
Sex	male	8.00 (7.00–9.00)	0.024†*	8.00 (6.00–10.00)	<0.001†*
	female	8.00 (7.00–8.00)		9.00 (7.00–10.00)	
BMI# ($N = 1,990$) [kg/m^2]	underweight	7.00 (6.00–8.00)	0.038‡*	8.00 (7.00–10.00)	0.039‡*
	normal	8.00 (6.00–8.00)		8.00 (7.00–10.00)	
	overweight	8.00 (7.00–8.00)		8.00 (7.00–10.00)	
	obesity	8.00 (7.00–9.00)		9.00 (8.00–10.00)	
Type of academic training	dental student	7.00 (6.00–8.00)	<0.001†*	9.00 (7.00–10.00)	0.129†
	dentist	8.00 (7.00–8.00)		8.00 (7.00–10.00)	
Specialty# ($N = 1,312$)	yes	8.00 (7.00–9.00)	0.035†*	8.00 (7.00–10.00)	0.922†
	no	8.00 (7.00–8.00)		8.00 (7.00–10.00)	
Place of origin	Mexico, Central America and the Caribbean	8.00 (7.00–9.00)	<0.001†*	8.50 (7.00–10.00)	0.782†
	South America	8.00 (7.00–8.00)		8.00 (7.00–10.00)	
Met someone with COVID-19	yes	8.00 (7.00–8.00)	<0.001†*	9.00 (7.00–10.00)	<0.001†*
	no	7.00 (6.00–8.00)		8.00 (7.00–10.00)	
COVID-19 information media	traditional	8.00 (7.00–8.00)	0.849†	8.00 (7.00–10.00)	0.390†
	virtual	8.00 (7.00–8.00)		8.00 (7.00–10.00)	

Data presented as *Me* (*Q1–Q3*). # responses with missing values; † Mann–Whitney *U* test; ‡ Kruskal–Wallis test; * statistically significant.

The number of days in the mandatory social isolation was associated with age ($p < 0.001$), the type of academic training ($p = 0.030$), having a specialty ($p < 0.001$), the place of origin ($p = 0.005$), and having met someone with COVID-19 ($p < 0.001$). The confinement level was associated with age ($p < 0.001$), sex ($p < 0.001$), BMI ($p < 0.001$), the type of academic training ($p < 0.001$), and having met someone with COVID-19 ($p = 0.002$) (Table 3).

Following the preventive measures for COVID-19 was associated with age ($p < 0.001$), the type of academic training ($p < 0.001$), having a specialty ($p = 0.004$), the place of origin ($p = 0.011$), and having met someone with COVID-19 ($p < 0.001$). The use of face masks, hand washing and social distancing were associated with age ($p < 0.001$), BMI ($p < 0.001$), the type of academic training ($p < 0.001$), the place of origin ($p = 0.003$), and having met someone with COVID-19 ($p = 0.002$) (Table 4).

an association between knowledge and age, in which the older group had significantly greater knowledge.[21] The tendency for older people to have more knowledge about the issues concerning COVID-19 may be due to the fact that older dentists are more aware of the need to continue education courses about infection control. As to the self-perceived level of concern regarding COVID-19, there was an association with sex and BMI, and it was lesser in those who did not meet anybody with COVID-19. It is important to keep in mind that the level of concern regarding COVID-19 is a subjective assessment that respondents provided on a Likert scale, in which the lowest value indicates no concern and the highest value represents great concern. This concern can be the cause of anxiety; Martina et al. found a relationship between concern and anxiety, with the majority of the concerned respondents being female and those at risk of treating a patient with a cough or suspected of being infected with COVID-19.[22] In the case of BMI, scientific evidence has shown that obesity

Table 3. Number of days in the mandatory social isolation and the confinement level of dental students and dentists during the mandatory social isolation ($N = 2,036$)

Variable		Number of days in the mandatory social isolation *Me* (*Q1–Q3*)	*p*-value	Confinement level *n* (%)				*p*-value
				I have not gone out any day	I have gone out very little	I have gone out frequently	I have gone out every day	
Age [years]	18–24	60.00 (60.00–70.00)	<0.001‡*	123 (20.30)	426 (70.30)	46 (7.59)	11 (1.82)	<0.001§*
	25–34	60.00 (47.00–70.00)		50 (6.68)	621 (82.91)	44 (5.87)	34 (4.54)	
	≥35	61.00 (56.00–85.00)		41 (6.02)	567 (83.26)	27 (3.96)	46 (6.75)	
Sex	male	60.00 (50.00–70.00)	0.082†	38 (6.30)	482 (79.93)	51 (8.46)	32 (5.31)	<0.001§*
	female	60.00 (55.00–72.00)		176 (12.28)	1,132 (79.00)	66 (4.61)	59 (4.12)	
BMI# ($N = 1,990$) [kg/m^2]	underweight	60.00 (60.00–70.00)	0.171‡	14 (16.87)	65 (78.31)	4 (4.82)	0 (0.00)	<0.001§§*
	normal	60.00 (54.00–70.00)		144 (11.59)	974 (78.42)	75 (6.04)	49 (3.95)	
	overweight	60.00 (54.00–75.00)		37 (6.97)	436 (82.11)	28 (5.27)	30 (5.65)	
	obesity	61.00 (54.00–85.00)		13 (9.70)	101 (75.37)	8 (5.97)	12 (8.96)	
Type of academic training	dental student	60.00 (60.00–70.00)	0.030†*	128 (17.68)	525 (72.51)	60 (8.29)	11 (1.52)	<0.001§*
	dentist	60.00 (52.00–75.00)		86 (6.55)	1,089 (83.00)	57 (4.34)	80 (6.10)	
Specialty# ($N = 1,312$)	yes	60.00 (54.00–80.00)	<0.001†*	57 (6.24)	767 (84.01)	41 (4.49)	48 (5.26)	0.213§
	no	60.00 (50.00–70.00)		29 (7.27)	322 (80.70)	16 (4.01)	32 (8.02)	
Place of origin	Mexico, Central America and the Caribbean	63.00 (60.00–79.50)	0.005†*	14 (8.54)	137 (83.54)	5 (3.05)	8 (4.88)	0.334§
	South America	60.00 (54.00–70.00)		200 (10.68)	1,477 (78.90)	112 (5.98)	83 (4.43)	
Met someone with COVID-19	yes	60.00 (50.00–85.00)	0.001†*	86 (8.72)	803 (81.44)	45 (4.56)	52 (5.27)	0.002§*
	no	60.00 (56.00–70.00)		128 (12.19)	811 (77.24)	72 (6.86)	39 (3.71)	
COVID-19 information media	traditional	62.00 (54.00–79.00)	0.698†	5 (8.20)	47 (77.05)	3 (4.92)	6 (9.84)	0.213§
	virtual	60.00 (55.00–72.00)		209 (10.58)	1,567 (79.34)	114 (5.77)	85 (4.30)	

responses with missing values; † Mann–Whitney U test; ‡ Kruskal–Wallis test; § χ^2 test; §§ χ^2 test for trend; * statistically significant.

Table 4. Following the preventive measures and protection recommendations for coronavirus disease 2019 (COVID-19) among dental students and dentists during the mandatory social isolation ($N = 2,036$)

Variable		Following the preventive measures for COVID-19					*p*-value	Use of face masks, hand washing and social distancing		*p*-value
		never	rarely	sometimes	usually	always		yes	no	
Age [years]	18–24	0 (0.00)	3 (0.50)	30 (4.95)	193 (31.85)	380 (62.71)	<0.001§§*	519 (85.64)	87 (14.36)	<0.001§§*
	25–34	2 (0.27)	1 (0.13)	11 (1.47)	230 (30.71)	505 (67.42)		677 (90.39)	72 (9.61)	
	≥35	0 (0.00)	2 (0.29)	6 (0.88)	138 (20.26)	535 (78.56)		660 (96.92)	21 (3.08)	
Sex	male	0 (0.00)	3 (0.50)	12 (1.99)	175 (29.02)	413 (68.49)	0.513§	548 (90.88)	55 (9.12)	0.773§
	female	2 (0.14)	3 (0.21)	35 (2.44)	386 (26.94)	1,007 (70.27)		1,308 (91.28)	125 (8.72)	
BMI# ($N = 1,990$) [kg/m^2]	underweight	0 (0.00)	1 (1.20)	1 (1.20)	26 (31.33)	55 (66.27)	0.360§	65 (78.31)	18 (21.69)	<0.001§*
	normal	2 (0.16)	4 (0.32)	33 (2.66)	342 (27.54)	861 (69.32)		1,122 (90.34)	120 (9.66)	
	overweight	0 (0.00)	1 (0.19)	7 (1.32)	155 (29.19)	368 (69.30)		499 (93.97)	32 (6.03)	
	obesity	0 (0.00)	0 (0.00)	6 (4.48)	29 (21.64)	99 (73.88)		127 (94.78)	7 (5.22)	
Type of academic training	dental student	2 (0.28)	3 (0.41)	30 (4.14)	237 (32.73)	452 (62.43)	<0.001§§*	621 (85.77)	103 (14.23)	<0.001§*
	dentist	0 (0.00)	3 (0.23)	17 (1.30)	324 (24.70)	968 (73.78)		1,235 (94.13)	77 (5.87)	
Specialty# ($N = 1,312$)	yes	0 (0.00)	2 (0.22)	12 (1.31)	205 (22.45)	694 (76.01)	0.004§§*	864 (94.63)	49 (5.37)	0.238§
	no	0 (0.00)	1 (0.25)	5 (1.25)	120 (30.08)	273 (68.42)		371 (92.98)	28 (7.02)	
Place of origin	Mexico, Central America and the Caribbean	0 (0.00)	0 (0.00)	0 (0.00)	34 (20.73)	130 (79.27)	0.011§§*	160 (97.56)	4 (2.44)	0.003§*
	South America	2 (0.11)	6 (0.32)	47 (2.51)	527 (28.15)	1,290 (68.91)		1,696 (90.60)	176 (9.40)	
Met someone with COVID-19	yes	0 (0.00)	3 (0.30)	20 (2.03)	231 (23.43)	732 (74.24)	<0.001§§*	919 (93.20)	67 (6.80)	0.002§*
	no	2 (0.19)	3 (0.29)	27 (2.57)	330 (31.43)	688 (65.52)		937 (89.24)	113 (10.76)	
COVID-19 information media	traditional	0 (0.00)	1 (1.64)	1 (1.64)	20 (32.79)	39 (63.93)	0.290§	53 (86.89)	8 (13.11)	0.233§
	virtual	2 (0.10)	5 (0.25)	46 (2.33)	541 (27.39)	1,381 (69.92)		1,803 (91.29)	172 (8.71)	

Data presented as *n* (%). # responses with missing values; § χ^2 test; §§ χ^2 test for trend; * statistically significant.

is a risk factor for mortality from COVID-19; therefore, people with this condition would be more concerned.[2] In the present study, the majority of respondents were women, which could cause greater variability in responses as compared to men, resulting in a statistical difference due to the heterogeneity of the sample. In addition, dentists may be concerned about the risk of infection from their coworkers or patients, which would generate the fear of infecting their family members.[23]

The mandatory social isolation was associated with professional formation. Vieira-Meyer et al. conducted a study in which they evaluated the development of clinical practices during the mandatory isolation in relation to the knowledge of COVID-19.[24] They found an inverse relationship; the greater the knowledge, the less compliance with social isolation restrictions.[24] No articles have been found on the association of isolation with the sociodemographic characteristics. However, Vieira-Meyer et al. reported that the knowledge of COVID-19 was closely related to a higher academic degree, and the latter was inversely related to the mandatory isolation.[24] Possibly, it was due to the type of specialized work the dentists performed. In that study, they worked in public entities; however, the study did not have a representative sample to generalize the findings.[24]

According to the preventive measures for COVID-19, such as the use of masks, hand washing and social distancing, it was reported that most of the population always followed the measures, with a predominance in the older group and in those who had met someone with COVID-19. These results are similar to those found in studies by Hleyhel et al. and Khader et al. with regard to preventive measures.[25,26] Gasparro et al. also found an association between following preventive measures and age as well as the clinical attention provided to possible COVID-19 patients.[27] Ahmed et al. found that due to clinical attention and explaining the measures to be taken in the event of a suspected COVID-19 case, the use of masks and the frequency of hand washing increased.[28] The results found in the present study are similar to those previously mentioned. This is because older people represent a population at greater risk from the imminent pandemic, which is why they adopt higher-level preventive measures.

In the same way, dentists who are exposed to aerosols while providing treatment to a patient with suspected infection can experience high levels of fear of COVID-19 contagion. The fear generates a strategic adaptation in order to find protection, which leads to the automatic monitoring of the established preventive measures. Dentistry has always elaborated protocols during the years of training on biosafety measures in clinical practice to avoid the spread of other infectious agents. Given the circumstances of the current pandemic and according to the WHO indications, hand hygiene is considered the first step to limit the spread of COVID-19. Therefore, hand washing with an alcohol-based sanitizer or soap and water should be performed before and after treating each patient. In addition, although surgical masks have always been used during dental treatment, the use of an N95 respirator is incorporated as a new measure, which is part of PPE. This is essential to prevent the inhalation of respiratory droplets.[26–29]

Limitations

This study has limitations. The 1st limitation is the type of validation of the survey. The 2nd limitation is the way in which the self-perceived level of knowledge and concern regarding COVID-19 were subjectively measured on the Likert scale. In addition, due to the temporality of the information, knowledge and attitudes may vary as research progresses. Another limitation is that the responses obtained did not cover all the countries in the region affected by the COVID-19 pandemic, especially when there are countries more affected than others. Furthermore, the policies, information and restrictions of each country are different, which could directly influence the responses of the participants. Consequently, the findings of this study should be interpreted carefully and not globalized.

Despite these limitations, the present findings may have significant implications for the public health policy. At the beginning of the pandemic, dentists showed great uncertainty due to the lack of knowledge of the disease, the risk of infection and mortality due to the high exposure they have when treating patients, which is greater than in most other professions.[10,19] In general, governments must allocate sufficient funds to develop adequate public policies for the implementation of preventive measures for health care personnel. Also, access to information for dental students and dentists on the preventive measures for COVID-19 in dental care can be improved through dissemination in different virtual information media. Therefore, it is necessary to identify and understand the preventive measures against COVID-19 in order to expand knowledge about biosafety standards and prevent the spread of the virus in dental practices.

Conclusions

Dental students and dentists followed the preventive measures for COVID-19 during the mandatory social isolation period in Latin America and the Caribbean in 2020. An association was found between the self-perceived level of knowledge about COVID-19, the self-perceived level of concern regarding COVID-19, the number of days in the mandatory social isolation, the confinement level, following the preventive measures for COVID-19, and the use of face masks, hand washing and social distancing with the sociodemographic characteristics and having met someone with COVID-19. Some differences in the level of significance according to the type of analyzed variables were also observed.

Ethics approval and consent to participate

The Research Ethics Committee at the Faculty of Dentistry of the University of Antioquia approved the study (Act 9-2020). The study followed the international standards for virtual surveys, and the respondents provided informed consent prior to completing the questionnaire. Confidentiality was guaranteed throughout the investigation process in accordance with the Declaration of Helsinki.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Brenda Melissa Flores-Quispe https://orcid.org/0000-0003-2076-0106
Renato Alejandro Ruiz-Reyes https://orcid.org/0000-0003-1480-5136
Roberto Antonio León-Manco https://orcid.org/0000-0001-9641-1047
Andrés Agudelo-Suárez https://orcid.org/0000-0002-8079-807X

References

1. He F, Deng Y, Li W. Coronavirus disease 2019 (COVID-19): What we know? *J Med Virol*. 2020;92(7):719–725. doi:10.1002/jmv.25766
2. Harapan H, Itoh N, Yufika A, et al. Coronavirus disease 2019 (COVID-19): A literature review. *J Infect Public Health*. 2020;13(5):667–673. doi:10.1016/j.jiph.2020.03.019
3. Hui DS, Azhar EI, Madani TA, et al. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health – the latest 2019 novel coronavirus outbreak in Wuhan, China. *Int J Infect Dis*. 2020;91:264–266. doi:10.1016/j.ijid.2020.01.009
4. Gorbalenya AE, Baker SC, Baric RS. Severe acute respiratory syndrome-related coronavirus: The species and its viruses – a statement of the Coronavirus Study Group. *bioRxiv*. 2020. doi:10.1101/2020.02.07.937862
5. Burki TK. Coronavirus in China. *Lancet Respir Med*. 2020;8(3):238. doi:10.1016/S2213-2600(20)30056-4
6. Pascarella G, Strumia A, Piliego C, et al. COVID-19 diagnosis and management: A comprehensive review. *J Intern Med*. 2020;288(2):192–206. doi:10.1111/joim.13091
7. Struyf T, Deeks JJ, Dinnes J, et al.; Cochrane COVID-19 Diagnostic Test Accuracy Group. Signs and symptoms to determine if a patient presenting in primary care or hospital outpatient settings has COVID-19 disease. *Cochrane Database Syst Rev*. 2020.7;7(7):CD013665. doi:10.1002/14651858.CD013665
8. Durán-Ojeda G. Preclinical simulation courses in dental education with COVID-19 pandemic [in Spanish]. *Odovtos – Int J Dent Sci*. 2020;22(2):11–13. doi:10.15517/IJDS.2020.41496
9. Luo M, Guo L, Yu M, Jiang W, Wang H. The psychological and mental impact of coronavirus disease 2019 (COVID-19) on medical staff and general public – a systematic review and meta-analysis. *Psychiatry Res*. 2020;291:113190. doi:10.1016/j.psychres.2020.113190
10. Gambarini G, Galli M, Gambarini E, et al. Fine aerosols and perceived risk of COVID-19 among Italian dental practitioners: An experimental survey. *J Contemp Dent Pract*. 2020;21(6):599–603. PMID:33025925.
11. Passarelli PC, Rella E, Manicone PF, Garcia-Godoy F, D'Addona A. The impact of the COVID-19 infection in dentistry. *Exp Biol Med (Maywood)*. 2020;245(11):940–944. doi:10.1177/1535370220928905
12. Villani FA, Aiuto R, Paglia L, Re D. COVID-19 and dentistry: Prevention in dental practice, a literature review. *Int J Environ Res Public Health*. 2020;17(12):4609. doi:10.3390/ijerph17124609
13. Estrich CG, Mikkelsen M, Morrissey R, et al. Estimating COVID-19 prevalence and infection control practices among US dentists. *J Am Dent Assoc*. 2020;151(11):815–824. doi:10.1016/j.adaj.2020.09.005
14. Luzzi V, Ierardo G, Bossù M, Polimeni A. COVID-19: Pediatric oral health during and after the COVID-19 pandemic. *Int J Paediatr Dent*. 2021;31(1):20–26. doi:10.1111/ipd.12737
15. Odeh ND, Babkair H, Abu-Hammad S, Borzangy S, Abu-Hammad A, Abu-Hammad O. COVID-19: Present and future challenges for dental practice. *Int J Environ Res Public Health*. 2020;17(9):3151. doi:10.3390/ijerph17093151
16. Burki T. COVID-19 in Latin America. *Lancet Infect Dis*. 2020;20(5):547–548. doi:10.1016/S1473-3099(20)30303-0
17. Ríos A. Number of confirmed cases of the novel coronavirus (COVID-19) in Latin America and the Caribbean, by country [in Spanish]. Statista. https://es.statista.com/estadisticas/1105121/numero-casos-COVID-19-america-latina-caribe-pais/. Accessed January 30, 2021.
18. León-Manco RA, Agudelo-Suárez AA, Armas-Vega A, et al. Perceived stress in dentists and dental students of Latin America and the Caribbean during the mandatory social isolation measures for the COVID-19 pandemic: A cross-sectional study. *Int J Environ Res Public Health*. 2021;18(11):5889. doi:10.3390/ijerph18115889
19. Gambarini E, Galli M, Di Nardo D, et al. A survey on perceived COVID-19 risk in dentistry and the possible use of rapid tests. *J Contemp Dent Pract*. 2020;21(7):718–722. PMID:33020352.
20. Habas K, Nganwuchu C, Shahzad F, et al. Resolution of coronavirus disease 2019 (COVID-19). *Expert Rev Anti Infect Ther*. 2020;18(12):1201–1211. doi:10.1080/14787210.2020.1797487
21. Kinariwala N, Samaranayake L, Perera I, Patel Z. Knowledge, awareness and perceptions of coronavirus disease 2019 (COVID-19) in a cohort of Indian dental professionals: A questionnaire-based study. *Preprints*. 2020:2020050442. doi:10.20944/preprints202005.0442.v1
22. Martina S, Amato A, Rongo R, Caggiano M, Amato M. The perception of COVID-19 among Italian dentists: An orthodontic point of view. *Int J Environ Res Public Health*. 2020;17(12):4384. doi:10.3390/ijerph17124384
23. Sezgin GP, Çapan BS. Assessment of dentists' awareness and knowledge levels on the novel coronavirus (COVID-19). *Braz Oral Res*. 2020;34:e112. doi:10.1590/1807-3107bor-2020.vol34.0112
24. Vieira-Meyer APGF, Coutinho MB, Santos HPG, Saintrain MV, Candeiro GTdeM. Brazilian primary and secondary public oral health attention: Are dentists ready to face the COVID-19 pandemic? *Disaster Med Public Health Prep*. 2020:1–8. doi:10.1017/dmp.2020.342
25. Hleyhel M, Haddad C, Haidar N, Charbachy M, Saleh N. Determinants of knowledge and prevention measures towards COVID-19 pandemic among Lebanese dentists: A cross sectional survey. *BMC Oral Health*. 2021;21(1):241. doi:10.1186/s12903-021-01599-9
26. Khader Y, Al Nsour M, Al-Batayneh OB, et al. Dentists' awareness, perception, and attitude regarding COVID-19 and infection control: Cross-sectional study among Jordanian dentists. *JMIR Public Health Surveill*. 2020;6(2):e18798. doi:10.2196/18798
27. Gasparro R, Scandurra C, Maldonato NM, et al. Perceived job insecurity and depressive symptoms among Italian dentists: The moderating role of fear of COVID-19. *Int J Environ Res Public Health*. 2020;17(15):5338. doi:10.3390/ijerph17155338
28. Ahmed MA, Jouhar R, Ahmed N, et al. Fear and practice modifications among dentists to combat novel coronavirus disease (COVID-19) outbreak. *Int J Environ Res Public Health*. 2020;17(8):2821. doi:10.3390/ijerph17082821
29. Meng L, Hua F, Bian Z. Coronavirus disease 2019 (COVID-19): Emerging and future challenges for dental and oral medicine. *J Dent Res*. 2020;99(5):481–487.doi:10.1177/0022034520914246

Cognizance, adverse effects and motivation regarding COVID-19 vaccination amongst health care professionals: A cross-sectional study

Shivani Sachdeva[A,B], Harish Saluja[C–F], Amit Mani[B]

Department of Periodontology, Rural Dental College, Pravara Institute of Medical Sciences, Loni, India

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):13–19

Address for correspondence
Shivani Sachdeva
E-mail: dr.shivani19@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on August 11, 2021
Reviewed on January 2, 2022
Accepted on January 12, 2022

Published online on February 3, 2022

Abstract

Background. Coronavirus disease 2019 (COVID-19) vaccines are currently at the forefront of India's fight against the pandemic. They have been shown to prevent the disease or reduce its severity, following 2 doses given at an interval of 6–8 weeks.

Objectives. The present cross-sectional survey was carried out on 1,145 health care practitioners in order to recognize and evaluate the adverse effects of vaccination as well as knowledge, motivation and attitudes with regard to vaccines amongst the respondents after the 1st dose of vaccination.

Material and methods. An anonymous survey was carried out among different age and gender groups in the medical colleges situated in the state of Maharashtra, India. The participants' responses were collected with the use of the Google Forms platform through sending a URL link to the questionnaire. The validity of the questionnaire was pilot-tested and the obtained Cronbach's alpha value was 0.80. Amongst the 1,145 participants, 92.2% of the respondents received COVISHIELD™ and 7.8% received COVAXIN®. The subjects were further scrutinized in 4 different age groups:18–27 years (73.0%); 28–37 years (14.8%); 38–47 years (9.6%); and above 47 years (2.5%).

Results. Adverse effects were common in the age group of 18–27 years and were observed significantly more frequently in females than males. A very common symptom was pain at the site of injection with an occurrence of 85.2%. Fever was present in 62.6% of the respondents, with 39.1% having low-grade fever and very few having high-grade fever.

Conclusions. Knowledge about adverse effects and what to expect after vaccination would help to educate people, dispel misinformation and reduce vaccination hesitancy. It can also help to promote awareness about the incidence of adverse effects and the safety of COVID-19 vaccines.

Keywords: vaccination, adverse effect, COVID-19 vaccine

Cite as
Sachdeva S, Saluja H, Mani A. Cognizance, adverse effects and motivation regarding COVID-19 vaccination amongst health care professionals: A cross-sectional study. *Dent Med Probl.* 2022;59(1):13–19. doi:10.17219/dmp/145757

DOI
10.17219/dmp/145757

Introduction

The coronavirus disease 2019 (COVID-19) pandemic sets great challenges, for which the world is only partially prepared. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) combines lethal pathogenicity with severe infectivity. While SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV) could be spread only by symptomatic patients, and thus could be repressed more easily, SARS-CoV-2 can be widely transmitted by both asymptomatic and pre-symptomatic individuals. Therefore, to curb the damage caused by COVID-19, primary efforts focus on confinement, physical distancing and vaccination.[1]

Two COVID-19 vaccines – COVAXIN® (Bharat Biotech, Hyderabad, India) and COVISHIELD™ (developed by the University of Oxford, UK/AstraZeneca, Cambridge, UK; manufactured by the Serum Institute of India, Pune, India) – are at the forefront for India in the fight against the disease. The Drugs Controller General of India has approved the viral vector vaccine Sputnik V, which was developed by the Gamaleya Research Institute of Epidemiology and Microbiology, Moscow, Russia. So, India is employing now the 3 vaccines in its large-scale COVID-19 vaccination drive, in which hundreds of millions doses are going to be administered.[2,3] Table 1 enumerates the ingredients of the COVISHIELD vaccine available in India. The approved vaccines and their efficacy are depicted in Fig. 1, though it is worth mentioning that many vaccines are in a trial stage.[4]

The survey had 2 major aims – firstly, to put forward the adverse effects of vaccination, and secondly, to motivate the frontline health care workers in India for vaccination. The particular goals were as follows:

– to study the spectrum of post-vaccination survey profiles for individual vaccines;
– to assess the immediate as well as late responses to the 1st dose of a COVID 19 vaccine;
– to assess motivation for the 2nd dose of a COVID 19 vaccine;
– to assess behavioral changes and attitudes toward the existing vaccines.

Table 1. Ingredients of the COVISHIELD vaccine

COVID-19 vaccine	Ingredients
COVISHIELD (Oxford/AstraZeneca)	l-histidine
	l-histidine hydrochloride monohydrate
	magnesium chloride hexahydrate
	polysorbate 80
	ethanol
	sucrose
	sodium chloride
	EDTA
	water for injection

COVID-19 – coronavirus disease 2019; EDTA – disodium edetate dihydrate.

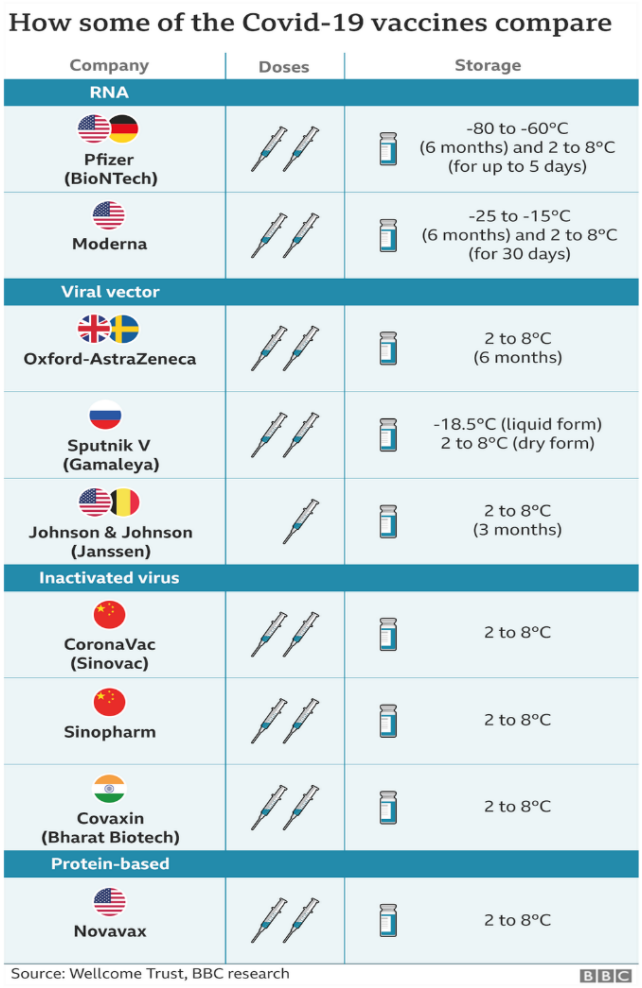

Fig. 1. Comparison of the various vaccines approved worldwide

The vaccination of health care professionals would not only protect them, but might also motivate the rest of the society to enthusiastically take part in vaccination.

The need of the hour is to identify common adverse effects, dissipate myths and abate vaccine uncertainty.

Material and methods

A descriptive, exploratory, anonymous cross-sectional survey for voluntary participation was carried out amongst medical and dental professionals. A total of 1,145 participants included the students, graduates, postgraduates, and staff working in the medical colleges situated in the state of Maharashtra, India, from February 25th to March 30th, 2021. The subjects were grouped based on the demographic variables of age and gender. There were 861 female and 284 male participants. Further, the subjects were scrutinized in 4 different age groups: 18–27 years (73.0%); 28–37 years (14.8%); 38–47 years (9.6%); and above 47 years (2.5%). No identifiable details were collected from the subjects.

An invitation message with a URL link to the online questionnaire was posted to the patricipants. The feedback

response rate was automatically saved. All responses were anonymous. The completion of the questionnaire required 4–5 min. The purpose of the survey was explained. Written informed consent was obtained during the introductory part of the survey; participation was entirely voluntary with the confidentiality of the participants preserved. Sending questionnaires to personal e-mail addresses was avoided to preserve the participants' anonymity and privacy. It was a self-administered questionnaire. Its internal consistency reliability and construct validity were determined with a Cronbach's alpha value of 0.80.

Statistical analysis

Descriptive statistics were used to assess the baseline characteristics. All quantitative variables were presented as mean and standard deviation ($M \pm SD$), and all qualitative variables as frequency – number and percentage (n (%)). The IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA), was used. The data was numerically coded and entered into the program. Both the descriptive statistics and the inferential statistics involving the χ^2 test were analyzed to compare the responses between the groups. For each test, a p-value of less than 0.05 was considered statistically significant. For continuous variables, the one-way analysis of variance (ANOVA)/the Kruskal–Wallis test were used to present the results.

Results

The present survey was carried out amongst the health care professionals in Maharashtra who got vaccinated for COVID-19. The total number of participants who could answer the questionnaire in the study was 1,145. The questionnaire was prepared in the form of 3 sets for the collection of data, and then the responses were assimilated. The participants were sent a link to the Google Forms platform, where the questionnaire was placed. The 1st set comprised questions about the demographic variables, including age and gender. The 2nd set of questions was related to the adverse effects experienced after vaccination, and the 3rd set of questions was related to motivation and the medications taken.

Adverse effects of COVID-19 vaccination in different age groups

Female health care professionals were greater in number and they were mostly aged 18–37 years. Adverse effects were common in the age group of 18–27 years and were observed significantly more frequently in females than males. The patient information brochure about the adverse effects of COVID-19 vaccination was read by 80% of the participants and 20% of the participants did not read the information prior to vaccination.

The graphical data presented in Fig. 2 shows the incidence of the post-vaccination symptoms amongst the participants in various age groups. The symptoms were observed in 78.5% of the participants from the age group of 18–27 years, followed by 72.4% of the participants aged 28–37 years and 53.6% of the participants aged 38–47 years. In the age group of 48 years and above, only 6.9% of the participants developed the symptoms.

Table 2 presents in detail the number and percentage of participants who experienced the symptoms and discomfort during work in various age groups after being administered the 1st dose of vaccination. Discomfort was observable mainly in the age group of 28–37 years (86.5% of the participants), followed by the participants from the age group of 38–47 years (57.3%). The results showed the importance of the assessment of discomfort during work, which could possibly be due to the presence of the symptoms after the vaccine administration.

It can be inferred from Table 2 that the onset of the symptoms was the slowest for the age group of 48 years and above; the group developed the symptoms in 13.40 h, while in the youngest age group, the symptoms occurred rapidly, within 8.45 h. Differences between the age groups in this respect were statistically significant.

It can also be inferred from Table 2 that the duration of the symptoms was the greatest for the youngest age group, but the difference was not statistically significant.

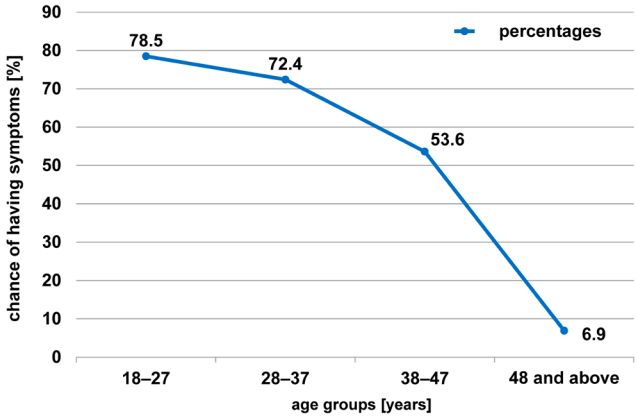

Fig. 2. Presence of the post-vaccination symptoms in different age groups

Adverse effects of COVID-19 vaccination in both gender groups

Table 3 shows the presence of the symptoms according to gender. The post-vaccination symptoms were more prevalent in females (79.2%) than males (47.9%). The onset of the symptoms was faster in males than females. The results were found to be statistically significant. Also, in terms of duration of the symptoms, the difference between the genders was clinically as well as statistically significant. Discomfort was also reported more frequently by females (69.1%) than males (35.6%) and the results were found to be statistically significant.

Table 2. Presence of the post-vaccination symptoms, their onset and duration, and the presence of discomfort during work after COVID-19 vaccination among the participants according to age

Variable		Age groups				*p*-value
		18–27 years	28–37 years	38–47 years	above 47 years	
Presence of symptoms	yes	656 (78.5)	123 (72.4)	59 (53.6)	2 (6.9)	0.001*
	no	180 (21.5)	47 (27.6)	51 (46.4)	27 (93.1)	
Onset of symptoms [h]		8.45 ±2.40	10.65 ±5.96	10.53 ±7.65	13.40 ±5.60	0.002*
Duration of symptoms [h]		28.20 ±15.20	25.78 ±13.80	20.82 ±10.30	20.90 ±7.21	0.081
Discomfort during work	yes	225 (26.9)	147 (86.5)	63 (57.3)	4 (13.8)	0.001*
	no	611 (73.1)	23 (13.5)	47 (42.7)	25 (86.2)	

Data presented as number (percentage) (*n* (%)) or mean ± standard deviation (*M* ±*SD*). * statistically significant.

Table 3. Presence of the post-vaccination symptoms, their onset and duration, and the presence of discomfort during work after COVID-19 vaccination among the participants according to gender

Variable		Gender		*p*-value
		male	female	
Presence of symptoms	yes	136 (47.9)	682 (79.2)	0.001*
	no	148 (52.1)	179 (20.8)	
Onset of symptoms [h]		8.15 ±7.65	13.26 ±5.25	0.001*
Duration of symptoms [h]		24.80 ±8.25	23.17 ±10.15	0.001*
Discomfort during work	yes	101 (35.6)	595 (69.1)	0.001*
	no	183 (64.4)	266 (30.9)	

Data presented as *n* (%) or *M* ±*SD*. * statistically significant.

Adverse effects of COVID-19 vaccination in the study population

The symptoms were categorized according to the fact sheet published by the Serum Institute of India, Pune, India, as very common, common and uncommon with regard to their relative incidence: >10%; ≤10%; and <1%, respectively.[5] The classification is discussed in detail below.

Very common and common symptoms

Very common symptoms included pain with the incidence of 85.2%. The incidence of mild or low-grade fever (99.0–102.2°F) was 39.1%, 19.1% of the participants had moderate-grade fever (102.3–104.0°F) and very few had high-grade fever (Fig. 3).

Figure 4 shows that the participants in majority did not seem to have any change in appetite after vaccination (74.8%). A meager percentage (20.0%) revealed decreased appetite, which could be due to the pertaining myalgia and weakness.

Headache was reported by 60.0% of the participants, drowsiness was observed in 52.2%, chills were present in 46.1%, while joint pain was present in 46.0% of the respondents. Also, 21.0% had a sore throat. A lump was one of the commonest symptoms in our survey and occurred in 14.0% of the respondents, and 12.2% had a running nose (Fig. 5).

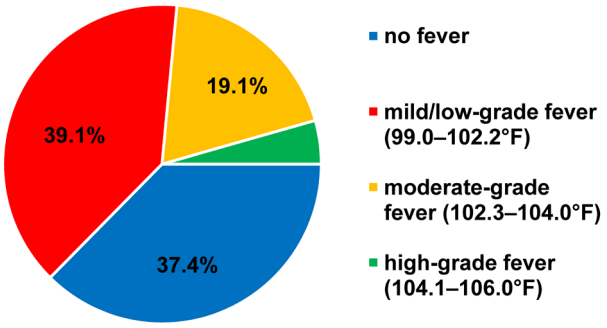

Fig. 3. Presence and grade of fever in the study participants

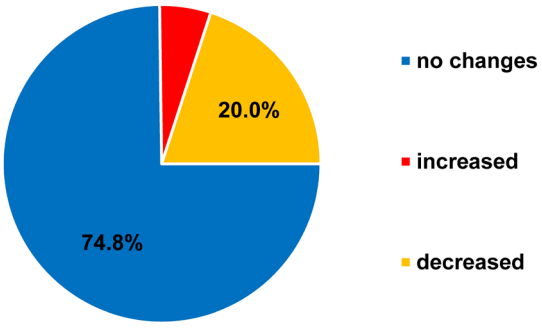

Fig. 4. Change in appetite in the study participants

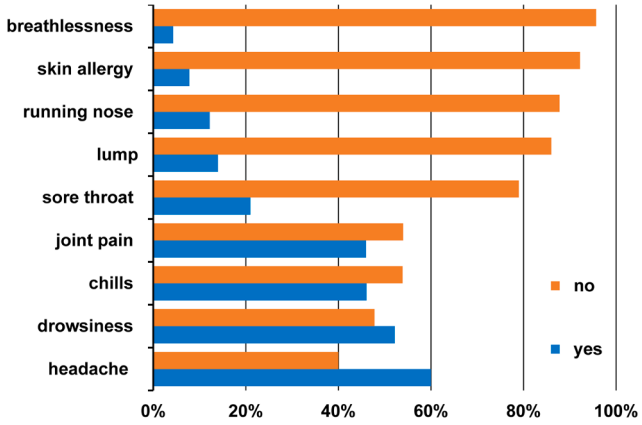

Fig. 5. Very common and common post-vaccination symptoms

Uncommon symptoms

The study showed that none of the participants had anaphylaxis. The post-vaccination symptoms either were absent or their presence was reported on the same day. After vaccination, none of the respondents had latent symptoms of herpes zoster or any other symptoms.

Behavioral changes regarding motivation and the application of medications

Motivation was increased after the 1st dose of vaccination and only 2.0% of the respondents refused to take the 2nd dose. A total of 98.5% were interested in motivating others for vaccination and 1.5% were in a dilemma. As many as 59.0% of the participants took 500 mg paracetamol after vaccination due to mild to moderate fever. Figure 6 depicts the day on which the post-vaccination symptoms started.

Discussion

Vaccines can be classified on the basis of the presence of whole viruses (live-attenuated or inactivated), viral vectors, nanoparticles or virus-like particles, subunit components, proteins/peptides, RNA, DNA, or live cells. COVID-19 vaccines tutor the immune system on how to espy and scuffle with the virus.[6]

To avoid the propagation of the pandemic, the basic reproduction number R0 (viral transmission) must remain below 1, signifying that each infected person transmits the disease on average to 1 new person. Otherwise, there is a calamitous exponential growth in the count of new infections. The spontaneous dissipation of the virus is dubious. Surplus flare-ups could be expected if the safety measures are sidelined. The world was poorly prepared for the 1st wave of the SARS-CoV-2 spread.[6] In order to limit the viral spread, restraint actions were undertaken, which halted further infections. The relaxation of the prevention rules

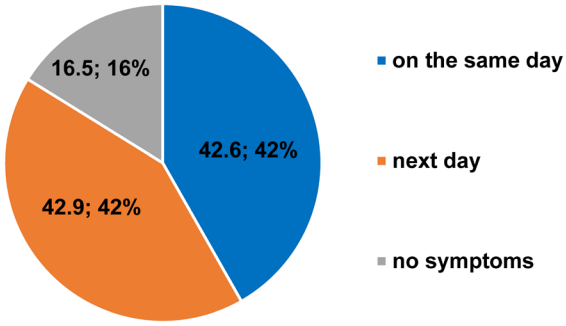

Fig. 6. Day on which the post-vaccination symptoms started

could contribute to new waves of the pandemic. Once vaccination is completed, it rapidly induces immunity in a pivotal percentage of the population, which is necessary for herd immunity. The immune response to SARS-CoV-2 consists in the activation of innate immunity as well as antigen-specific responses of T cells and B cells.[7]

Protection from a viral infection is mainly accomplished by the formation of virus-neutralizing antibodies. Humans gain potent immune protection after getting infected or vaccinated, which constitutes a fundamental principle for a wide variety of infections caused by viruses.

Broad immunization is notably beneficial as soon as "only" approx. 60–70% of the population have become immune. Inculcating touchstones regarding vaccination would taper off the viral spread.

Vaccine antigens

B cell/antibody targets

The available vaccines are mainly based on virus-neutralizing antibodies. Such antibodies usually block the interaction of the virus with its cellular receptor or prevent the conformational changes required for the melding of the virus with the cell membrane.

T cell targets

CD4 and CD8 T cells recognize and react with the SARS-CoV-2 antigens, contributing to immune protection, particularly by reducing disease severity. To some extent, it may also be due to the cross-reactive T cells induced by seasonal coronaviruses. T cells are perhaps less effective than the neutralizing antibodies in controlling the disease. Preventive anti-viral vaccines are favorable, as they induce antibodies that nullify viral particles in the extracellular space instantly after they enter the body and before the virus infects the host's cells. CD4 T helper cells strongly promote B cell responses and antibody production. Thus, both B cells and T cells are induced by vaccines concurrently.[7,8]

Adverse effects

Five types of adverse effects of immunization that are observed are vaccine product-related reaction, vaccine quality defect-related reaction, immunization error-related reaction, immunization anxiety-related reaction, and coincidental event.

With regard to the adverse effects of COVID-19 vaccines, the fact sheet for a vaccine recipient issued by the Serum Institute of India classifies the symptoms as very common, common and uncommon[5]:

– very common (affecting more than 1 in 10 people):
  - tenderness, pain, warmth, redness, itching, swelling, or bruising at the site of injection,
  - generally feeling unwell,
  - feeling tired (fatigue),
  - chills or feeling feverish,
  - headache,
  - feeling sick (nausea),
  - joint pain or muscle ache;

– common (affecting no more than 1 in 10 people):
  - a lump at the site of injection,
  - fever,
  - being sick (vomiting),
  - flu-like symptoms, such as high temperature, a sore throat, a running nose, a cough, and chills;

– uncommon (affecting 1 in 100 people):
  - feeling dizzy,
  - decreased appetite,
  - abdominal pain,
  - enlarged lymph nodes,
  - excessive sweating, itchy skin or a rash.

Despite knowing the possible side effects, and even the start of the 2nd wave of the COVID-19 pandemic, which is reported to be more deadly, there is still a lot of psychological stress among people with regard to vaccination. Therefore, the positive motivation of the whole society is a must.

Jayadevan et al. conducted a cross-sectional online survey that included questions pertaining to the immediate post-vaccination experience in Kerala, India.[9] The survey was performed during a 1-week period from January 29 to February 4, 2020, on 5,396 people. At least 1 post-vaccination symptom was reported among 65.9% of the participants. Fatigue (45%), myalgia (44%), fever (34%), headache (28%), local pain at the site of injection (27%), joint pain (12%), nausea (8%), and diarrhea (3%) were the most prevailing symptoms.[9] Also in the present study, mild fever was found in 39% of the participants, and the common adverse effects were more or less similar to those reported in the survey carried out in Kerala.

In the abovementioned study, the symptoms seemed to decrease with age,[9] which was similar to the findings of our study. The iteration of symptoms was 81% (3rd decade, 20–29 years), 80% (4th decade, 30–39 years), 68% (5th decade, 40–49 years), 58% (6th decade, 50–59 years), 45% (7th decade, 60–69 years), 34% (8th decade, 70–79 years), and 7% (9th decade, 80–90 years),[9] which is in accordance with the results of the present study, where the incidence of the post-vaccination symptoms was the greatest in the age group of 18–27 years, with a gradual decrease in incidence as the age of the participants increased.

Jayadevan et al. observed that the post-vaccination symptoms were reported more frequently by women (74.7%) as compared to men (58.6%) ($p < 0.001$).[9] The inferences of the present study correspond to the above in terms of gender; adverse effects were more prevalent in females than males. Among the vaccinated female participants, 79% developed the post-vaccination symptoms, while the percentage was 47% in the case of males. As reported in February 2021 by the Centers for Disease Control and Prevention (CDC), during the 1st month of the COVID 19 vaccine rollout, 72% of the reported side effects occurred in females.[10] Also, following the 2009–2010 H1N1 (swine flu) pandemic vaccine distribution, researchers found that 4 times as many women as men aged 20–59 years reported an allergic response to the H1N1 vaccine; the possible reason could be that the female sex hormones, estrogens, might be related to a stronger immune response.[10] Similar results were obtained in the present study, where 79% of females reported adverse effects.

Limitations

The limitation of the present study was the selection bias, as the target population were health care professionals. The sample size of the population was small. Also, the self-reported data could be the source of bias, as the respondents might overestimate as well as underestimate their perception of adverse effects on the scale, which might have affected the outcomes of the study. Moreover, more systematic reviews and randomized controlled trials are required to relate the adverse effects of vaccination to the demographic data.

Conclusions

The study participants presented adverse effects like fever, chills, joint pain, a sore throat, a lump at the site of injection, and a running nose, most of them resembling the symptoms of influenza. Their incidence was greater in females than males. The youngest age group (18–27 years) was most affected. Currently, there is no standard treatment for COVID-19 across the globe. Applying simple measures, such as hand washing with soap, disinfection, wearing masks, or using personal protective equipment (PPE), should be practiced on regular basis. Researchers have made great efforts to bring out the various vaccines across the globe within an astonishing time period of just 1 year. It is essential for the masses to get themselves

injected and get herd immunity against the virus so as to prevent its further spread. There is a need to motivate the population for vaccination to reduce the mortality and curb the condition.

The need of the hour is to develop vaccines that aim at the initiation of protective immune responses, principally via virus-neutralizing antibodies specific for SARS-CoV-2. Although at least 1–2 years will be required to make efficacious vaccines available globally, vaccination may still be the most rapid and economical strategy to achieve widespread immune protection. The so-called "herd immunity" has to be reached, deserting the virus to local chances for circulation. This will happen when a critical percentage (>90%) of the population is immune.

Future perspectives

Undoubtedly, trust is an elemental and feasibly modifiable factor in the successful uptake of COVID-19 vaccination. We should revise the exemplars we learned from earlier infectious disease outbreaks and public health emergencies, which included the diseases caused by human immunodeficiency virus (HIV), H1N1, SARS, MERS, and Ebola. These admonish us that the trustworthy sources of information and guidance are the axioms in the control of such outbreaks. It is essential to address the hesitancy related to vaccination. It is a voluminous, complicated and context-dependent endeavor that must be made simultaneously at international, national and communal stages. Clear and consistent communication by government officials is crucial to building public confidence in vaccination programs.

Ethics approval and consent to participate

The ethical approval was obtained from the institutional Research Ethics Committee (No. of approval: PIMS/DR/RDC/2020/35). The respondents provided informed consent prior to completing the questionnaire.

Data availability

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

Consent for publication

The subjects understood that their names and initials would not be published and due efforts would be made to conceal their identity, but anonymity could not be guaranteed.

ORCID iDs

Shivani Sachdeva https://orcid.org/0000-0003-2124-8120
Harish Saluja https://orcid.org/0000-0002-9511-083X
Amit Mani https://orcid.org/0000-0002-4433-7487

References

1. Sachdeva S, Saluja H, Mani A, Mani S, Chawla K, Mohammadi SN. Facial masks for COVID-19 pandemic an approach toward awareness among health practitioners. *Journal Head Neck Physicians Surg*. 2021;9(2):136–141. doi:10.4103/jhnps.jhnps_19_21
2. Sah R, Shrestha S, Mehta R, et al. AZD1222 (Covishield) vaccination for COVID-19: Experiences, challenges, and solutions in Nepal. *Travel Med Infect Dis*. 2021;40:101989. doi:10.1016/j.tmaid.2021.101989
3. Bharat Biotech. Bharat Biotech announces phase 3 results of COVAXIN®: India's first COVID-19 vaccine demonstrates interim clinical efficacy of 81%. 2021. https://www.bharatbiotech.com/images/press/covaxin-phase3-efficacy-results.pdf. Accessed March 3, 2021.
4. Tripathi A. At 3 million doses, biggest tranche of Sputnik V vaccines lands in India. 2021. https://www.ndtv.com/india-news/at-3-million-doses-biggest-tranche-of-sputnik-v-vaccines-lands-in-india-2453619. Accessed June 11, 2021.
5. Fact sheet on COVISHIELD™. 2021. https://www.seruminstitute.com/pdf/covishield_fact_sheet.pdf. Accessed March 30, 2021.
6. Triggle N. COVID: Can we really jab our way out of lockdown? 2021. https://www.bbc.com/news/health-55488724. Accessed January 5, 2021.
7. Menachery VD, Gralinski LE, Mitchell HD, et al. Combination attenuation offers strategy for live attenuated coronavirus vaccines. *J Virol*. 2018;92(17):e00710-18. doi:10.1128/JVI.00710-18
8. Speiser DE, Bachmann MF. COVID-19: Mechanisms of vaccination and immunity. *Vaccines (Basel)*. 2020;8(3):404. doi:10.3390/vaccines803040
9. Jayadevan R, Shenoy R, Ts A. Survey of symptoms following COVID-19 vaccination in India. *medRxiv*. 2021. doi:10.1101/2021.02.08.21251366
10. Gee J, Marquez P, Su J, et al. First month of COVID-19 vaccine safety monitoring – United States, December 14, 2020–January 13, 2021. *MMWR Morb Mortal Wkly Rep*. 2021;70(8):283–288. doi:10.15585/mmwr.mm7008e3

Acceptance of SARS-CoV-2 vaccination and the associated factors among dental health care professionals: A cross-sectional survey

Hafsa Qabool[A–F], Fatima Hamid[A–C,F], Rashna Hoshang Sukhia[A,C,E,F]

Section of Dentistry (Orthodontics), Department of Surgery, The Aga Khan University Hospital, Karachi, Pakistan

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):21–26

Address for correspondence
Rashna Hoshang Sukhia
E-mail: rashna.aga@aku.edu

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on October 28, 2021
Reviewed on December 27, 2021
Accepted on January 3, 2022

Published online on February 9, 2022

Abstract

Background. One of the greatest inventions of the 21st century is the development of vaccines against the life-threatening pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Whenever a new medication or treatment modality is introduced globally, it is accompanied by anxiety in the general public and among health care professionals.

Objectives. The aim of the study was to explore factors that may influence the acceptance of COVID-19 vaccination among dental health care professionals, as they are the first subgroup in the population to receive the vaccine.

Material and methods. A survey-based cross-sectional study was conducted on 164 health care professionals (general dentists, dental specialists with 2 years of experience after graduation and dental assistants). Data was collected by sending a URL link to the hard- and soft-copy questionnaire on Google Forms through all social media platforms. The questionnaire had 2 sections – the 1st part concerned the demographic details and the 2nd part was designed to assess the acceptance of SARS-CoV-2 vaccination among dental health care professionals and the related factors. The normality of the data was assessed with the Shapiro–Wilk test. The Cox regression algorithm was applied to evaluate the factors associated with the acceptance of SARS-CoV-2 vaccination.

Results. Out of 164 participants, 85.37% showed a positive attitude toward vaccination and only 7.32% of dental health care professionals were not willing to get vaccinated; out of them, 5 were males and 7 were females. Those who refused to get vaccinated included 3.6% of general dentists, 21.1% of dental specialists and 11.7% of dental assistants. The complications of major concern were fever, myalgia and the lethargic condition immediately after vaccination.

Conclusions. A small percentage of health care professionals declined to get vaccinated against COVID-19 and the main reason was uncertainty about the associated side effects. The respondents were mostly concerned about such side effects as fever, myalgia and the lethargic condition immediately after vaccination.

Keywords: SARS-CoV-2, dentist, vaccine, acceptance, health care professional

Cite as
Qabool H, Hamid F, Sukhia RH. Acceptance of SARS-CoV-2 vaccination and the associated factors among dental health care professionals: A cross-sectional survey. *Dent Med Probl.* 2022;59(1):21–26. doi:10.17219/dmp/145491

DOI
10.17219/dmp/145491

Introduction

December 2019 brought along a global humanitarian crisis due to the rapid spread of a disease caused by different variants of coronavirus – coronavirus disease 2019 (COVID-19) – with 87% chances of cross-infection and a 4% mortality rate.[1] Policy-making organizations and health care setups declared an emergency situation to avoid the spread of the infection.[2] Every country imposed strict guidelines and protocols to control the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Wearing a face mask and social distancing were made elemental protocols worldwide to minimize the rate of spread of the coronavirus through airborne droplets.[3] Despite all these preventive measures, SARS-CoV-2 spread rapidly in 2020 and policy-making organizations were worried about halting this viral web.[4] Hence, the development and deployment of COVID-19 vaccines was considered to be the most promising health care fortification step to mitigate the rapid spread of the SARS-CoV-2 infection.[5]

One of the greatest inventions of the 21st century is the development of vaccination against life-threatening COVID-19.[6] Current research is focused mainly on the trials of the newly developed vaccines against this deadly virus and their effects on the overall human health.[7] Whenever a new medication or treatment modality is introduced globally, it is accompanied by anxiety in the general public and among health care professionals.[8] The situation is quite challenging in the case of SARS-CoV-2 vaccines due to an already heightened level of stress and apprehension.[9] This anxiety is associated with the alarming spread of the infection and a gradual increase in the mortality rate associated with the disease. At the end of December 2020, a mass vaccination program was initiated. The World Health Organization (WHO) collaborated with different companies worldwide to complete the critical steps of manufacturing safe and efficacious vaccines.[10] One of the factors that greatly influenced the attitude of the general population toward COVID-19 vaccines was the misinformation being spread by anti-vaccination activists.[11] Approximately 7% of the population of Saudi Arabia did not get vaccinated against influenza due to the fear of getting sick.[6] This rate of acceptance was attributed to a multitude of factors, including location, the educational status and social behaviors.[6]

A survey conducted by Thunström et al. to explore the acceptance of vaccination revealed that around 20% of the population in the USA declined a COVID-19 vaccine.[12] The most valid reasons for this non-acceptance were the possible side effects and vaccine ineffectiveness against the disease. Thus, it is imperative to explore factors that may influence the acceptance of vaccination among dental health care professionals, as they are highly exposed to SARS-CoV-2, working close to the oral cavity. Also, health care professionals along with the elderly population are among the initial subsets of the population to receive the vaccine allocated by the WHO.[10]

Therefore, this study aimed to assess the rate of acceptance of COVID-19 vaccination and the factors affecting the acceptance among dental health care professionals.

Material and methods

An analytical survey-based cross-sectional study was conducted after obtaining an approval from the institutional Research Ethics Committee (No. of approval: 2021-6233-17837). Data was collected by sending a URL link to the questionnaire on Google Forms through all the approachable social media platforms (e-mail, WhatsApp and Facebook Messenger). A modified version of the validated questionnaire of Posse et al.[13] was used in this study. It comprised 2 sections. The 1st section was based on informed consent and concerned the demographic information about the study participants. The 2nd section was framed to assess the acceptance of COVID-19 vaccination among dental health care professionals along with its associated factors. The questionnaire was then assessed by 3 dental health care professionals for its face validity.

The sample size of the study was calculated with the OpenEpi software, v. 3.01 (https://www.openepi.com/Menu/OE_Menu.htm). Since, according to the findings of Wang et al.,[11] the anticipated proportion of the acceptance of vaccination among dental health care professionals was kept at 89.5%, and a level of significance was set at 5%, precision at 5% and a design effect at 1, a sample size of 160 dental health care professionals was required for this study with an inflation of 10%.

The study questionnaire was sent to general dentists, dental specialists with 2 years of experience after graduation and dental assistants. Five reminders were sent to the non-responders before excluding them from the study. Moreover, the participants were asked to forward the Google form link to other dental health care professionals; in this way, data was collected by means of a simplified snowball sampling technique.

Statistical analysis

The data was analyzed using the statistical software for data science Stata®, v. 12.0 (StataCorp, College Station, USA). Descriptive statistics for continuous variables were reported as mean and standard deviation ($M \pm SD$), as the data was normally distributed. Nominal data was reported as percentage frequency or as number and percentage (n (%)). The unadjusted and adjusted prevalence ratios of the factors influencing the acceptance of vaccination were assessed using the Cox regression algorithm with a 95% confidence interval (*CI*).

Results

Demographics

A summary of the descriptive analysis with the percentage frequencies of the participants' responses is shown in Table 1. Our sample included 81 males and 83 females, with 28 general dentists, 76 dental specialists and 60 dental assistants. Among a total of 164 participants, 85.37% showed a positive attitude toward vaccination. Only 7.32% of dental health care professionals were not willing to get vaccinated; out of them, 5 were males and 7 were females. Among health care professionals, we found that 3.6% of general dentists, 21.1% of dental specialists and 11.7% of dental assistants refused vaccination (Table 2). Overall, 32.5% of the participants were concerned about fever, myalgia and lethargy, 28.0% were anxious about an allergic reaction to SARS-CoV-2 vaccination, 26.5% were worried about headaches and neurological complications, and the remaining 13.0% of the respondents were worried about respiratory complications (Table 1).

Table 1. Acceptance of vaccination among 3 groups of dental health care professionals ($N = 164$)

Factors influencing the acceptance of SARS-CoV-2 vaccination			Dental health care professionals		
			general dentists	dental specialists	dental assistants
Q.1	Do you want to receive the vaccine as soon as it becomes available?	yes	96.4	78.9	88.3
		no	3.6	21.1	11.7
Q.2	If not, what is your reason for not getting vaccinated?	not an effective option	21.1	10.3	8.6
		unsafe	31.6	30.8	8.6
		vaccine created in rush	31.6	43.6	40.0
		immunity will develop	15.8	0.0	34.3
		other	0.0	15.4	8.6
Q.3	Where did you get information on SARS-CoV-2 vaccination?	electronic/social media	85.7	73.7	70.3
		publications/conferences/seminars	10.7	21.0	29.7
		people/relatives	3.6	5.3	0.0
Q.4	How would you rate your knowledge about the COVID-19 pandemic?	good	7.1	61.8	55.0
		moderate	35.7	36.8	43.3
		poor	57.2	1.3	1.7
Q.5	How much has this pandemic affected your daily routine?	significantly	92.9	82.5	91.7
		insignificantly	7.1	17.5	8.3
Q.7	Do you think you will get infected with the virus after getting vaccinated?	yes	46.4	59.2	56.7
		no	3.6	9.2	8.3
		not sure	50.0	31.6	35.0
Q.8	Did you ever refuse any vaccination in the past?	yes	0.0	7.9	6.7
		no	100.0	92.1	93.3
Q.9	Do you think SARS-CoV 2 vaccination will be an effective way to minimize and control this infection?	yes	46.4	59.2	56.7
		no	3.6	9.2	8.3
		not sure	50.0	31.6	35.0
Q.10	Is the price of the vaccine an important factor in deciding whether to get vaccinated or not?	yes	50.0	28.9	35.0
		no	50.0	63.2	53.3
		not sure	0.0	7.9	11.7
Q.11	Is the vaccination schedule an essential factor in making a decision regarding getting vaccinated?	yes	50.0	52.6	65.0
		no	50.0	42.1	16.7
		not sure	0.0	5.3	18.3
Q.12	Do you think the SARS-CoV 2 vaccine will have specific side effects?	yes	96.4	68.3	68.4
		no	3.6	13.3	10.5
		not sure	0.0	18.3	21.1
Q.13	Which side effects are you particularly worried about?	allergic reaction	21.4	38.2	18.3
		neurological disturbances	14.3	22.4	10.0
		respiratory disturbances	28.6	13.0	6.7
		fever/myalgia/lethargy	35.7	26.4	65.0

Data presented as percentage values. SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; COVID-19 – coronavirus disease 2019.

Table 2. Descriptive statistics ($N = 164$)

Dental health care professionals	Gender *n* (%)		Age [years] (*M* ±*SD*)	Experience [years] (*M* ±*SD*)	Percentage of acceptance [%]	Percentage of unacceptance [%]
	male	female				
General dentists	9 (32.1)	19 (67.9)	31.05 ±6.56	4.87 ±3.34	96.4	3.6
Dental specialists	37 (48.7)	39 (51.3)	36.09 ±8.14	8.48 ±6.31	78.9	21.1
Dental assistants	35 (58.3)	25 (41.7)	31.32 ±4.87	8.47 ±5.65	88.3	11.7

M = 81; F = 83; M – males; F – females; *n* – number; *M* – mean; *SD* – standard deviation.

Acceptance of SARS-CoV-2 vaccination

Using the Cox regression algorithm, we found a statistically non-significant difference in the acceptance of vaccination between general dentists, dental specialists and dental assistants. As many as 96.4% of general dentists, 78.9% of dental specialists and 88.3% of dental assistants were willing to receive the vaccine. However, 3.6% of general dentists, 21.1% of dental specialists and 11.7% of dental assistants refused to be vaccinated. Among those who refused to get vaccinated, 50.0% were not sure if the vaccination was safe and 33.3% had doubts as to the development of vaccines; they were not sure if the regulatory bodies had approved the vaccine after the required research trials, as shown in Table 3. We found that there was a statistically non-significant difference in the acceptance of vaccination between genders (Table 3).

Table 3. Factors influencing the acceptance of vaccination among dental health care professionals ($N = 164$)

Variable		Hazard ratio	95% *CI*	*p*-value	R^2
Male gender		1.05	0.75, 1.46	0.750	0.75
Designation (general dentists as reference)	dental specialists	0.80	0.54, 1.19	0.290	0.57
	dental assistants	0.86	0.55, 1.34	0.510	
If you don't want to receive the vaccine, what is the reason?	not an effective option	1.26	0.85, 1.88	0.230	0.68
	unsafe	1.26	0.66, 2.43	0.470	
	vaccine created in rush	0.90	0.51, 1.61	0.730	
	immunity will develop	1.01	0.57, 1.77	0.950	
Where did you get information on SARS-CoV-2 vaccination?	electronic/social media	1.41	0.57, 3.46	0.440	0.67
	publications/conferences/seminars	1.28	0.49, 3.31	0.610	
How would you rate your knowledge about the COVID-19 pandemic?	moderate	0.82	0.29, 2.28	0.710	0.16
	poor	0.86	0.31, 2.35	0.770	
How much has this pandemic affected your daily routine?	insignificantly	1.08	1.02, 1.20	0.050*	1.08
Do you think you will get infected with the virus after getting vaccinated?	no	1.02	0.71, 1.45	0.910	0.35
	not sure	1.20	0.65, 2.21	0.550	
Did you ever refuse any vaccination in the past?	no	1.06	0.53, 2.07	0.870	0.03
Do you think SARS-CoV 2 vaccination will be an effective way to minimize and control this infection?	no	1.13	0.67, 1.91	0.630	0.30
	not sure	1.31	0.38, 4.50	0.660	
Is the price of the vaccine an important factor in deciding whether to get vaccinated or not?	no	1.79	0.81, 0.71	0.140	0.27
Is the vaccination schedule an essential factor in making a decision regarding getting vaccinated?	no	1.08	0.59, 1.99	0.780	0.10
Do you think the SARS-CoV 2 vaccine will have specific side effects?	no	0.82	0.54, 1.25	0.360	0.66
Which side effects are you particularly worried about?	allergic reaction	1.05	0.61, 0.51	0.850	0.79
	neurological disturbances	0.93	0.51, 1.71	0.830	
	respiratory disturbances	0.88	0.46, 1.69	0.720	
	fever/myalgia/lethargy	1.99	0.57, 2.05	0.790	

Cox regression algorithm model; *CI* – confidence interval; * statistically significant ($p \leq 0.05$).

Discussion

Since the outbreak of COVID-19, efforts have been constantly made to control the pandemic and curb the rapid spread of coronavirus,[12–14] and since the advent of vaccines against SARS-CoV-2 to limit cross-infection, many surveys have been performed to assess the acceptance of vaccination.[15] The majority of those surveys focused on the general population.[16,17] On the Asian continent, the vaccine was first introduced to the subset of health care professionals. Hence, health care professionals can be an authentic source of evidence regarding factors that influence the acceptance of SARS-CoV-2 vaccination.[18] Therefore, a survey like this may provide essential information to policy-making organizations and institutions to target the apprehension and motivate the rest of the population for vaccination.

In this survey, we found that only 7.32% of the participants were not willing to get vaccinated, 7.31% were hesitant about getting vaccinated, while as many as 85.37% of the participants accepted the vaccine. The present results are in contrast to a survey-based study conducted by Murhekar et al. in the USA, where 33.3% of the participants refused SARS-CoV-2 vaccination.[19] The initial survey conducted in May 2020 reported a 47% rate of vaccination refusal.[19] This gradual decrease in the percentage of health care professionals refusing SARS-CoV-2 vaccination may be due to the fact that awareness is increasing. The decreasing percentage also reflects the motivation of health care professionals to play a role in limiting the spread of cross-infection.[20] Hesitation about getting vaccinated was found to be multifactorial. The cultural fears, side effects and general myths related to this disease make people uncertain about vaccination. According to the findings of Cascini et al., attitudes toward vaccination were also closely related to the initiatives taken by the government and the awareness campaigns conducted by the doctors.[21] Health care professionals base their decisions on scientific evidence and the results of trials. These long-term trials have increased trust in the safety and effectiveness of vaccination.[21]

It is perceived that the acceptance of SARS-CoV-2 vaccination increases along with the level of experience and designation, as specialists were more willing to get vaccinated as compared to dental assistants.[22] This may be due to the fact that dental assistants and dental staff with less experience lack valid information on vaccination.[22] However, it is undeniable that overall, health care professionals faced a wave of uncertainty in this pandemic, which may have led them to spread rumors and myths regarding SARS-CoV-2 vaccination.

This survey found that almost 73% of the participants gained awareness with regard to SARS-CoV-2 vaccination via electronic and social media. Hence, electronic and social media may also be helpful in disseminating authentic information on SARS-CoV-2 vaccination. The WHO has taken an important initiative to address the myths regarding vaccination by answering the most commonly asked queries of the general population and uploading them on its website.[23] We suggest that policy-making organizations should make use of electronic and social media, and take key steps to clear up misinformation among the population. This would increase the number of vaccinated people and eventually limit the spread of cross-infection.

Limitations

The limitations of this study are a survey-based cross-sectional study design with a non-probability snowball sampling technique and a small sample size. However, we believe that a long-term study should be conducted.

Conclusions

There was a small percentage of health care professionals refusing SARS-CoV-2 vaccination. The main reason was uncertainty about side effects, the most expected being fever, myalgia and lethargy following vaccination.

Ethics approval and consent to participate

The ethical approval was obtained from the institutional Research Ethics Committee (No. of approval: 2021-6233-17837). The respondents provided informed consent prior to completing the questionnaire.

Data availability

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

Consent for publication

The subjects understood that their names and initials would not be published, and due efforts would be made to conceal their identity**,** but anonymity could not be guaranteed.

ORCID iDs

Hafsa Qabool https://orcid.org/0000-0002-8968-2014
Fatima Hamid https://orcid.org/0000-0002-1279-355X
Rashna Hoshang Sukhia https://orcid.org/0000-0001-9210-6432

References

1. Kaur H, Kochhar AS, Gupta H, Singh G, Kubavat A. Appropriate orthodontic appliances during the COVID-19 pandemic: A scoping review. *J Oral Biol Craniofac Res*. 2020;10(4):782–787. doi:10.1016/j.jobcr.2020.10.014
2. Lee TVN, Fowler PV, Williams JC, Ellis P, Atack NE, Ireland AJ. Orthodontics at times of national emergency: Past and current crises Part 2. COVID-19: Implications for clinical practice. *Orthod Update*. 2020;13(3):108–116. doi:10.12968/ortu.2020.13.3.108

3. Dalewski B, Palka L, Kiczmer P, Sobolewska E. The impact of SARS-CoV-2 outbreak on the Polish dental community's standards of care – a six-month retrospective survey-based study. *Int J Environ Res Public Health*. 2021;18(3):1281–1284. doi:10.3390/ijerph18031281
4. Marshall S, Duryea M, Huang G, et al. COVID-19: What do we know? *Am J Orthod Dentofacial Orthop*. 2020;158(5):e53–e62. doi:10.1016/j.ajodo.2020.08.010
5. Adly AS, Adly AS, Adly MS. Approaches based on artificial intelligence and the Internet of intelligent things to prevent the spread of COVID-19: Scoping review. *J Med Internet Res*. 2020;22(8):e19104. doi:10.2196/19104
6. Perillo L, Nucci L, Yitschaky O, Carrino G, Carrino R, Chaushu S. Returning to the orthodontic practice amid COVID-19 crisis. *Stoma Edu J*. 2020;7(3):209–215. doi:10.25241/stomaeduj.2020.7(3).art.7
7. Zahran S, Wright N. A review of COVID-19 and the implications for orthodontic provision in England. *Orthod Update*. 2020;13(3):117–124. doi:10.12968/ortu.2020.13.3.117
8. Hegde MN, Parmar G, Logani A, et al. Dental practice management during COVID-19 times – now and beyond. *Int J Clin Pract*. 2021;75(9):e14251. doi:10.1111/ijcp.14251
9. Bakaeen LG, Masri R, AlTarawneh S, et al. Dentists' knowledge, attitudes, and professional behavior toward the COVID-19 pandemic: A multisite survey of dentists' perspectives. *J Am Dent Assoc*. 2021;152(1):16–24. doi:10.1016/j.adaj.2020.09.022
10. World Health Organization. Coronavirus disease (COVID-19) pandemic. 2020. https://www.who.int/emergencies/diseases/novel-coronavirus-2019. Accessed September 15, 2021.
11. Wang J, Jing R, Lai X, et al. Acceptance of COVID-19 vaccination during the COVID-19 pandemic in China. *Vaccines (Basel)*. 2020;8(3):482. doi:10.3390/vaccines8030482
12. Thunström L, Ashworth M, Finnoff D, Newbold SC. Hesitancy towards a COVID-19 vaccine. *Ecohealth*. 2021;18(1):44–60. doi:10.1007/s10393-021-01524-0
13. Posse JL, van Harten MT, Mac Giolla Phadraig C, et al. The impact of the first wave of the COVID-19 pandemic on providing special care dentistry: A survey for dentists. *Int J Environ Res Public Health*. 2021;18(6):2970. doi:10.3390/ijerph18062970
14. Day CJ, Price R, Sandy JR, Ireland AJ. Inhalation of aerosols produced during the removal of fixed orthodontic appliances: A comparison of 4 enamel cleanup methods. *Am J Orthod Dentofacial Orthop*. 2008;133(1):11–17. doi:10.1016/j.ajodo.2006.01.049
15. Renukaradhya GJ, Isloor S, Rajasekhar M. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. *Vet Microbiol*. 2020;90(1–4):183–195. doi:10.1016/S0378-1135(02)00253-5
16. Qian H, Zheng X. Ventilation control for airborne transmission of human exhaled bio-aerosols in buildings. *J Thorac Dis*. 2018;10(Suppl 19):S2295–S2304. doi:10.21037/jtd.2018.01.24
17. Guo YR, Cao QD, Hong ZS, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. *Mil Med Res*. 2020;7(1):11. doi:10.1186/s40779-020-00240-0
18. Holshue ML, DeBolt C, Lindquist S, et al.; Washington State 2019-nCoV Case Investigation Team. First case of 2019 novel coronavirus in the United States. *N Engl J Med*. 2020;382(10):929–936. doi:10.1056/NEJMoa2001191
19. Murhekar MV, Bhatnagar T, Selvaraju S, et al. Prevalence of SARS-CoV-2 infection in India: Findings from the national serosurvey, May–June 2020. *Indian J Med Res*. 2020;152(1&2):48–60. doi:10.4103/ijmr.IJMR_3290_20
20. American Dental Association, MouthHealthy™. 2020. Infection control. https://www.mouthhealthy.org/en/az-topics/i/infection-control. Accessed September 15, 2021.
21. Cascini F, Pantovic A, Al-Ajlouni Y, Failla G, Ricciardi W. Attitudes, acceptance and hesitancy among the general population worldwide to receive the COVID-19 vaccines and their contributing factors: A systematic review. *EClinicalMedicine*. 2021;40:101113. doi:10.1016/j.eclinm.2021.101113
22. Qabool H, Sukhia RH, Fida M. Knowledge and awareness of dental specialists, general dentists and dental assistants regarding SARS-CoV-2. *Dent Med Probl*. 2021;58(3):285–290. doi:10.17219/dmp/134964
23. World Health Organization, Regional Office for the Eastern Mediterranean. COVID-19 situation in the Region. Situation as of October 4, 2021. http://www.emro.who.int/health-topics/corona-virus/index.html. Accessed September 15, 2021.

Knowledge, attitude and practice among dental practitioners with regard to overcoming the barriers created by personal protective equipment in the COVID-19 era and delivering effective dental treatment: A questionnaire-based cross-sectional study

Vallari Malandkar[1,A–F], Sheetal Choudhari[2,A–F], Dheeraj Kalra[3,A–F], Parmeet Banga[4,A–F]

[1] General dentist, Private practice, Navi Mumbai, India
[2] Department of Oral Pathology and Microbiology, Yerala Medical Trust's Dental College and Hospital, Navi Mumbai, India
[3] Department of Public Health Dentistry, Yerala Medical Trust's Dental College and Hospital, Navi Mumbai, India
[4] Department of Prosthodontics, Yerala Medical Trust's Dental College and Hospital, Navi Mumbai, India

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):27–36

Address for correspondence
Vallari Malandkar
E-mail: vallarimalandkar16@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on June 26, 2021
Reviewed on September 12, 2021
Accepted on September 17, 2021

Published online on February 10, 2022

Cite as
Malandkar V, Choudhari S, Kalra D, Banga P. Knowledge, attitude and practice among dental practitioners with regard to overcoming the barriers created by personal protective equipment in the COVID-19 era and delivering effective dental treatment: A questionnaire-based cross-sectional study. *Dent Med Probl.* 2022;59(1):27–36. doi:10.17219/dmp/142357

DOI
10.17219/dmp/142357

Abstract

Background. In the wake of the coronavirus disease 2019 (COVID-19) pandemic, dental professionals are at high risk of contracting the virus owing to their close proximity to patients. Using personal protective equipment (PPE) is necessary to avoid being infected as well as to avoid being the source of infection. Apart from physical limitations, also communication and work efficiency are affected by the barriers created by PPE.

Objectives. This study was conducted to assess knowledge, attitude and practice regarding the challenges faced by dental practitioners in India due to the use of PPE as well as to discuss the ways of overcoming these barriers by dentists.

Material and methods. A cross-sectional study was conducted during a period of 1 month. A Google Forms questionnaire was sent out; it included 12 questions regarding the use of PPE, changes in the diet and the work routine, the side effects of PPE, effects on communication and work efficiency, and the patients' attitude toward PPE. The obtained data was subjected to the statistical analysis with the use of the IBM SPSS Statistics for Windows software, v. 26.0. For all statistical tests, $p < 0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study of 80%.

Results. A total of 390 dentists completed the questionnaire. The study revealed that 85% of the respondents agreed that wearing PPE affected their work efficiency and 89% experienced difficulty in communication. The majority of the participants experienced side effects, like profuse sweating, breathlessness, headaches, and skin irritation.

Conclusions. It was proven that the current use of PPE not only makes communication harder, but also elevates anxiety among patients. Dentists have adapted themselves by switching to other means of communication, such as sending instructions by means of text messages/telemedicine, as well as taking breaks between patients, switching to a healthier diet, and exercising regularly, thus helping to minimize the adverse effects of PPE.

Keywords: dentists, dental anxiety, COVID-19, personal protective equipment (PPE), side effects of PPE

Introduction

The coronavirus disease 2019 (COVID-19) pandemic has tremendously affected the dental profession. Dentists are at high risk of contracting and transmitting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes COVID-19, alongside paramedics, nurses and other health care workers. The virus is mainly present in the nasopharyngeal and salivary secretions of infected patients, and is spread primarily through respiratory droplets, aerosols and fomites. Dental treatment requires from the dentist to be in close proximity to the patient's mouth and throat, which makes the dental personnel highly exposed to the virus, and contributes to the transmission of the virus to other staff members and patients. Many dental procedures, such as using headpieces and ultrasonic instruments, generate aerosols, increasing the likelihood of spreading the virus, which makes dentistry one of the most high-risk professions.[1] COVID-19 has not only increased the fear of aerosol contamination during dental treatment, but also caused the fear of close contacts.[2] The use of disinfectants and personal protective equipment (PPE) remains imperative for maintaining proper working conditions and preventing further transmission. Personal protective equipment minimizes exposure to the contaminated body fluids, reducing the risk of infection.[3] It creates a physical barrier between the pathogenic organisms and the operator, thus preventing any droplets from settling on the operator's skin. However, dentists are facing a new set of challenges, both physical and mental, from wearing PPE as they adapt to provide quality dental care in the COVID-19 era.

Along with the physical limitations created by PPE, communication is also affected due to the already increased levels of anxiety and stress created during this health care crisis.[4] Understanding the dentist becomes significantly more difficult for patients and the dental assistant due to the added layers of PPE along with the surrounding noise of the compressor, suction, fans, and other equipment. Consequently, it is hard for the patient to follow the operator's instructions during treatment. Personal protective equipment, which includes gloves, gowns, shoe covers, head covers, masks, respirators, face shields, and goggles, hides facial expressions, the main tool for displaying emotions. Smiling, one of the easiest and most pleasing ways to connect with other person, is no longer an option. Masks muffle voices, making it more difficult to catch every word and infer the emotion associated with it. In addition to these barriers, there are several side effects associated with the use of PPE, resulting in physical and emotional exhaustion, which in turn affects the dentist's work efficiency.

This survey focuses on the assessment of the common concerns of practicing dentists in the COVID-19 era and was designed to study the overall effects of PPE on a dental setup.

The aim of the study was to assess the common concerns among dentists related to their practice in the COVID-19 era, to assess their use of PPE on a daily basis, to determine if the pandemic has in any way changed their work routine, and to assess any side effects and difficulty in communication created by the use of PPE.

Material and methods

Study settings and participants

This questionnaire-based cross-sectional study was conducted over a period of 1 month between February 1 and March 5, 2021. The participants consisted of practicing dentists from various parts of India with varying experience, age, sex, and specialty. The study participants were approached by the principal investigator.

Sample size

The sample size was estimated using the OpenEpi software, v. 3.01 (https://www.openepi.com/Menu/OE_Menu.htm), and the 'sample size for frequency in a population' formula. A p-value <0.05 was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study of 80%; a total sample size of 390 was derived.

Data collection

A structured, self-explanatory questionnaire was created in English on the Google Forms platform. It consisted of a brief introduction to the study, followed by 2 sections. Section I collected the demographic data, and section II consisted of 12 open- and closed-ended questions regarding the participants' knowledge, attitude and practice with regard to the use of PPE, changes in their diet and work routine, the side effects of PPE, and effects on communication and work efficiency (Table 1). The study participants were approached by the principal investigator via personal connections and the Internet/e-based technologies (e.g., online platforms and e-mail). A pilot study was initially conducted with 10 participants. According to their responses and feedback, the following changes were made:

– the questions were divided into 2 sections – section I consisted of questions related to the sociodemographic data, while section II consisted of questions related to the use of PPE in a dental setup;
– multiple option checkboxes were added wherever necessary.

The questionnaire was then sent to the study participants and their responses were kept confidential. Timely reminders were sent as well. Participation was completely voluntary and all the participants could opt out of the study by not completing the questionnaire.

Table 1. Questionnaire used in the study

	No.	Questions	Answer options
Section I: Questions related to the sociodemographic data	1.	Name	
	2.	Age [years]	a) 20–30 b) 31–40 c) 41–50 d) above 50
	3.	Sex	a) male b) female
	4.	Specialty	a) general dentistry b) endodontics c) oral medicine and radiology d) oral pathology e) oral surgery f) orthodontics g) pediatric dentistry h) periodontics i) prosthodontics j) public health dentistry
	5.	Experience [years]	a) <5 b) 5–10 c) 10–15 d) 15–20 e) >20
	6.	Type of practice	a) institution/college b) private clinic c) both
Section II: Questions related to the use of PPE in a dental setup	1.	What components of PPE do you use most commonly?	a) gown b) mask/respirator c) shoe cover d) head cover e) eye protection f) face shield g) all of the above
	2.	According to you, which is the most effective mask?	a) N95 mask b) FFP2 mask c) surgical mask d) respirator
	2a.	What type of mask do you use?	a) N95 mask b) FFP2 mask c) surgical mask d) respirator
	3.	How many times do you remove your PPE during a normal workday?	a) never b) 2–3 times c) 4–5 times d) after each patient
	4.	Do you take lunch/ water breaks?	a) yes b) no
	5.	As compared to pre-COVID-19 times, have you changed your food intake pattern?	a) yes b) no
	6.	As compared to pre-COVID-19 times, how have you changed your work routine to accommodate to the use of PPE?	a) reducing working hours b) completing all patients at one go, and then changing PPE c) taking breaks and changing PPE d) reducing the number of workdays e) any other
	7.	Has PPE affected your work efficiency?	a) yes b) no
	7a.	If yes, how?	a) more time being spent per patient b) impaired vision c) feeling of exhaustion/fatigue d) any other
	8.	What side effects do you experience due to the use of PPE?	a) headaches b) breathlessness c) skin irritation/oily skin d) profuse sweating e) hoarse voice f) dry throat g) blocked/stuffy nose h) dry mouth i) any other
	9.	Do you feel there is difficulty in communication due to the use of PPE?	a) yes b) no
	9a.	If yes, amongst whom?	a) between the dentist and the patient b) between the dentist and the dental assistant
	9b.	How do you overcome this?	
	10.	Do you think the patient is more anxious seeing you in PPE?	a) yes b) no c) don't know
	10a.	If yes, what do you do to alleviate the patient's anxiety?	
	11..	How do you explain the treatment plan to the patient?	a) on the dental chair while wearing PPE b) at the front desk after doffing PPE c) by clicking pictures and explaining them to the patient over WhatsApp d) any other
	12.	How do you give post-operative instructions and medications to the patient?	a) on the dental chair while wearing PPE b) at the front desk, by the receptionist c) over phone/WhatsApp d) any other

PPE – personal protective equipment; COVID-19 – coronavirus disease 2019.

Statistical analysis

The data was compiled in a Microsoft Office Excel spreadsheet (v. 2019; Microsoft Corporation, Redmond, USA). The data was subjected to the statistical analysis with the use of the IBM SPSS Statistics for Windows software, v. 26.0 (IBM Corp., Armonk, USA). Descriptive statistics like number and percentage (n (%)) were used for categorical data. Numerical data was expressed as mean and standard deviation ($M \pm SD$). The comparison of frequencies for the categories of variables between the groups was made using the χ^2 test. For all statistical tests, $p < 0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study of 80%.

Results

This survey had a sample size of 390 dentists who completed the questionnaire (201 general dentists and 189 specialists). More than half of the participants (n = 273; 70.00%) were between 20 and 30 years of age, with 269 (68.97%) having worked less than 5 years. Among all the participants, 158 (40.51%) were male and 232 (59.49%) were female. A total of 174 (44.62%) respondents were practicing at an institution/college and 104 (26.67%) were practicing at a private clinic. The distribution of specialists was as follows: 45 prosthodontists; 33 orthodontists; 23 pedodontists; 19 endodontists; 18 oral pathologists; 15 oral surgeons; 15 periodontists; 14 radiologists, and 7 public health dentists. These details are presented in Table 2.

Table 2. Demographic details of the participants (N = 390)

Demographic details		n (%)
Age [years]	20–30	273 (70.00)
	31–40	67 (17.18)
	41–50	26 (6.67)
	above 50	24 (6.15)
Sex	male	158 (40.51)
	female	232 (59.49)
Experience [years]	<5	269 (68.97)
	5–10	49 (12.56)
	10–15	30 (7.69)
	15–20	10 (2.56)
	>20	32 (8.21)
Specialty	general dentistry	201 (51.54)
	prosthodontics	45 (11.53)
	orthodontics	33 (8.46)
	pediatric dentistry	23 (5.90)
	endodontics	19 (4.87)
	oral pathology	18 (4.61)
	oral surgery	15 (3.85)
	periodontics	15 (3.85)
	oral medicine and radiology	14 (3.59)
	public health dentistry	7 (1.79)
Type of practice	institution/college	174 (44.62)
	private clinic	104 (26.67)
	both	112 (28.71)

n – number.

Approximately ⅓ of the participants (n = 142; 36.41%) used a gown, a mask/respirator, a head cover/hood, and a face shield as part of their PPE on a daily basis. Among the respondents, 283 (72.56%) agreed that N95 masks were the most effective ones, and 224 (57.44%) used an N95 mask regularly. It was found that 163 (41.79%) of the participants removed their PPE 2–3 times a day, while 109 (27.95%) never removed their PPE during a normal workday.

As per the responses recorded, 133 (34.10%) participants did not take any lunch/water breaks between patients. As compared to pre-COVID times, 117 (30.00%) respondents changed their food intake pattern. The survey found that 40 (10.26%) dentists decided to have healthier foods, such as freshly prepared home-cooked meals, including vegetables, fruits and eggs (i.e., a fibrous, proteinaceous, vitamin-rich diet). There were 30 (7.69%) who answered that they skipped meals or had delayed meals, whereas 28 (7.18%) maintained a strict diet routine and hygiene, and avoided in-between snacking.

According to 331 (84.87%) participants, wearing PPE had affected their work efficiency, while 59 (15.13%) responded that it had not. Just over ⅓ of the participants (n = 137; 35.13%) had to spend more time per patient, had their vision impaired and felt exhaustion. It was found that 89% of the respondents had difficulty in communication due to the use of PPE, while 11% stated that they had no difficulty. Among the participants, 38% responded to the open-ended question on how they overcome this difficulty in communication. For 21.54% of the dentists, this involved repeating loudly, 9.74% preferred other means of communication (like providing instructions via text messages or calls, writing on a piece of paper, or using a microphone with a small speaker), 4.62% preferred communicating before/after the procedure, and 2.56% removed the mask or the face shield for a moment. Among the respondents, 28% agreed that the patient seemed more anxious upon seeing the dentist in PPE. When asked how they alleviate this anxiety, 41 (10.51%) stated that they had a conversation and reassured the patient, 23 (5.90%) explained the importance of wearing PPE to the patient and 14 (3.59%) lightened the mood with a joke. The majority of the participants (n = 231; 59.23%) responded that they explained the treatment plan and gave post-operative instructions to the patient on the dental chair while wearing their PPE, 84 (21.54%) provided this information at the front desk

after doffing their PPE, and some used a mobile phone/WhatsApp. These results are presented in Table 3.

Highly statistically significant differences were observed in the response rates for certain categories ($p < 0.010$), with higher frequencies for the response 'female' (Table 4), for the participants falling within the age group of 20–30 years (Table 5) and for the participants working in the institutional/dental hospital type of practice (Table 6). When the response rates were compared with regard to the number of years of experience, there was a higher frequency for the participants with less than 5 years of experience ($p < 0.010$) (Table 7). When comparing specialties, there was a statistically higher response rate from the participants practicing as general dentists ($p < 0.010$) (Table 8).

Figure 1 presents the responses regarding side effects: 287 participants experienced profuse sweating; 203 participants experienced breathlessness; 168 participants suffered from headaches; 145 participants had a dry mouth; 113 participants experienced a dry throat; 111 respondents experienced skin irritation or oily skin; 83 participants had a blocked/stuffy nose; and 28 participants experienced a hoarse voice.

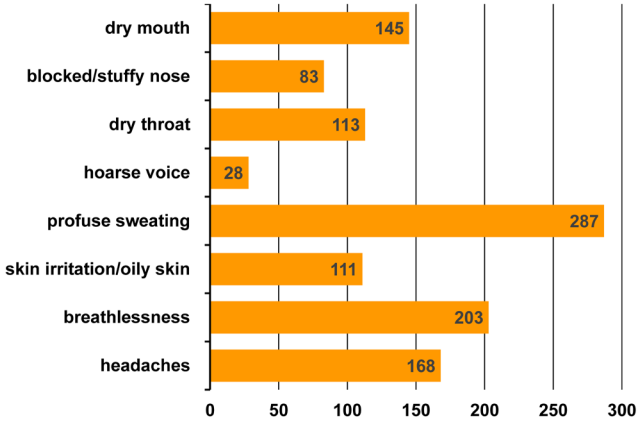

Fig. 1. Side effects of personal protective equipment (PPE)

Table 3. Overall responses to the questionnaire

Question	Response	*n* (%)
What components of PPE do you use most commonly?	gown, mask/respirator, head cover/hood, face shield	142 (36.41)
	gown, mask/respirator, face shield	100 (25.64)
	all of the above	102 (26.15)
According to you, which is the most effective mask?	N95 mask	283 (72.56)
	respirator	85 (21.79)
What type of mask do you use?	N95 mask	224 (57.44)
	N95 mask, surgical mask	98 (25.13)
How many times do you remove your PPE during a normal workday?	never	109 (27.95)
	2–3 times	163 (41.79)
	after each patient	98 (25.13)
As compared to pre-COVID-19 times, how have you changed your food intake pattern?	healthier foods, like freshly prepared home-cooked meals, including vegetables, fruits, eggs (a fibrous, proteinaceous, vitamin-rich diet)	40 (10.26)
	prefer having food at home and not at the clinic	19 (4.87)
	skipping meals or having delayed meals	30 (7.69)
	maintaining strict routine and hygiene, avoiding in-between snacking	28 (7.18)
As compared to pre-COVID-19 times, how have you changed your work routine to accommodate to the use of PPE?	reducing working hours	118 (30.26)
	completing all patients at one go, and then changing PPE	127 (32.56)
	taking breaks and changing PPE	105 (26.9)2
How has PPE affected your work efficiency?	more time being spent per patient, impaired vision, feeling of exhaustion/fatigue	137 (35.13)
Amongst whom do you feel difficulty in communication the most?	between the dentist and the patient, between the dentist and the dental assistant	208 (53.33)
How do you overcome this?	repeating loudly	84 (21.54)
	other means of communication	38 (9.74)
	removing the mask or the face shield for a moment	10 (2.56)
What do you do to alleviate the patient's anxiety?	having a conversation and reassuring the patient	41 (10.51)
	explaining the importance of wearing PPE	23 (5.90)
	lightening the mood by cracking a joke	14 (3.59)
How do you explain the treatment plan and provide post-operative instructions to the patient?	on the dental chair while wearing PPE	231 (59.23)
	at the front desk after doffing PPE	84 (21.54)

Table 4. Comparison of the responses according to sex

Question	Response	Sex		Total	*p*-value
		female	male		
What components of PPE do you use most commonly?	gown, mask/respirator, head cover/hood, face shield	86	56	142	<0.010*
	all of the above	60	42	102	
According to you, which is the most effective mask?	N95 mask	183	100	283	<0.010*
	respirator	42	43	85	
What type of mask do you use?	N95 mask	133	91	224	<0.010*
	N95 mask, surgical mask	66	32	98	
How many times do you remove your PPE during a normal workday?	never	65	44	109	<0.010*
	2–3 times	107	56	163	
As compared to pre-COVID-19 times, how have you changed your work routine to accommodate to the use of PPE?	reducing working hours	71	47	118	<0.010*
	completing all patients at one go, and then changing PPE	68	59	127	
	taking breaks and changing PPE	68	37	105	
How has PPE affected your work efficiency?	more time being spent per patient, impaired vision, feeling of exhaustion/fatigue	98	39	137	<0.010*
How do you overcome difficulty in communication?	repeating loudly	46	38	84	<0.010*
	other means of communication	20	18	38	
What do you do to alleviate the patient's anxiety?	having a conversation and reassuring the patient	28	13	41	<0.010*
	explaining the importance of wearing PPE	9	14	23	
How do you explain the treatment plan and provide post-operative instructions to the patient?	on the dental chair while wearing PPE	127	104	231	<0.010*
	at the front desk after doffing PPE	54	30	84	

Data presented as number (*n*). * statistically significant.

Table 5. Comparison of the responses according to age

Question	Response	Age [years]				Total	*p*-value
		20–30	31–40	41–50	above 50		
What components of PPE do you use most commonly?	gown, mask/respirator, head cover/hood, face shield	105	22	4	11	142	<0.010*
	all of the above	57	23	18	4	102	
According to you, which is the most effective mask?	N95 mask	205	49	18	11	283	<0.010*
	respirator	51	17	8	9	85	
What type of mask do you use?	N95 mask	133	56	24	11	224	<0.010*
	N95 mask, surgical mask	89	1	0	8	98	
How many times do you remove your PPE during a normal workday?	never	72	25	8	4	109	<0.010*
	2–3 times	112	21	19	11	163	
As compared to pre-COVID-19 times, how have you changed your work routine to accommodate to the use of PPE?	reducing working hours	67	30	16	5	118	<0.010*
	completing all patients at one go, and then changing PPE	94	12	9	12	127	
	taking breaks and changing PPE	84	14	2	5	105	
How has PPE affected your work efficiency?	more time being spent per patient, impaired vision, feeling of exhaustion/fatigue	113	16	6	2	137	<0.010*
How do you overcome difficulty in communication?	repeating loudly	49	22	4	9	84	<0.010*
	other means of communication	21	8	8	1	38	
What do you do to alleviate the patient's anxiety?	having a conversation and reassuring the patient	34	5	2	0	41	<0.010*
	explaining the importance of wearing PPE	11	8	4	0	23	
How do you explain the treatment plan and provide post-operative instructions to the patient?	on the dental chair while wearing PPE	155	43	16	17	231	<0.010*
	at the front desk after doffing PPE	64	14	2	4	84	

Data presented as number (*n*). * statistically significant.

Table 6. Comparison of the responses according to type of practice

Question	Response	Type of practice			Total	*p*-value
		institution/ college	private clinic	both		
What components of PPE do you use most commonly?	gown, mask/respirator, head cover/hood, face shield	75	29	38	142	<0.010*
	all of the above	38	26	38	102	
According to you, which is the most effective mask?	N95 mask	141	63	79	283	<0.010*
	respirator	22	34	29	85	
What type of mask do you use?	N95 mask	87	59	78	224	<0.010*
	N95 mask, surgical mask	51	35	12	98	
How many times do you remove your PPE during a normal workday?	never	31	33	45	109	<0.010*
	2–3 times	74	57	32	163	
As compared to pre-COVID-19 times, how have you changed your work routine to accommodate to the use of PPE?	reducing working hours	50	30	38	118	<0.010*
	completing all patients at one go, and then changing PPE	42	53	32	127	
	taking breaks and changing PPE	64	15	26	105	
How has PPE affected your work efficiency?	more time being spent per patient, impaired vision, feeling of exhaustion/fatigue	83	28	26	137	<0.010*
How do you overcome difficulty in communication?	repeating loudly	21	32	31	84	<0.010*
	other means of communication	14	12	12	38	
What do you do to alleviate the patient's anxiety?	having a conversation and reassuring the patient	20	9	12	41	<0.010*
	explaining the importance of wearing PPE	7	6	10	23	
How do you explain the treatment plan and provide post-operative instructions to the patient?	on the dental chair while wearing PPE	86	82	63	231	<0.010*
	at the front desk after doffing PPE	42	16	26	84	

Data presented as number (*n*). * statistically significant.

Table 7. Comparison of the responses according to the number of years of experience

Question	Response	Experience [years]					Total	*p*-value
		<5	5–10	10–15	15–20	>20		
What components of PPE do you use most commonly?	gown, mask/respirator, head cover/hood, face shield	101	21	8	1	11	142	<0.010*
	all of the above	57	15	10	8	12	102	
According to you, which is the most effective mask?	N95 mask	205	31	22	6	19	283	<0.010*
	respirator	47	19	6	4	9	85	
What type of mask do you use?	N95 mask	139	30	28	8	19	224	<0.010*
	N95 mask, surgical mask	82	8	0	0	8	98	
How many times do you remove your PPE during a normal workday?	never	68	23	6	2	10	109	<0.010*
	2–3 times	116	14	16	4	13	163	
As compared to pre-COVID-19 times, how have you changed your work routine to accommodate to the use of PPE?	reducing working hours	69	18	16	8	7	118	<0.010*
	completing all patients at one go, and then changing PPE	88	16	2	2	19	127	
	taking breaks and changing PPE	84	10	6	0	5	105	
How has PPE affected your work efficiency?	more time being spent per patient, impaired vision, feeling of exhaustion/fatigue	105	22	6	0	4	137	<0.010*
How do you overcome difficulty in communication?	repeating loudly	41	17	15	0	11	84	<0.010*
	other means of communication	24	3	6	0	5	38	
What do you do to alleviate the patient's anxiety?	having a conversation and reassuring the patient	34	2	3	0	2	41	<0.010*
	explaining the importance of wearing PPE	13	2	6	2	0	23	
How do you explain the treatment plan and provide post-operative instructions to the patient?	on the dental chair while wearing PPE	157	27	16	8	23	231	<0.010*
	at the front desk after doffing PPE	60	14	6	0	4	84	

Data presented as number (*n*). * statistically significant.

Table 8. Comparison of the responses according to the specialty

Question	Response	Specialty										Total	*p*-value
		general dentistry	prosthodontics	orthodontics	pediatric dentistry	endodontics	oral pathology	oral surgery	periodontics	oral medicine and radiology	public health dentistry		
What components of PPE do you use most commonly?	gown, mask/respirator, head cover/hood, face shield	68	15	9	21	6	6	4	8	1	4	142	<0.010*
What type of mask do you use?	N95 mask	99	37	14	18	12	12	6	12	10	4	224	<0.010*
As compared to pre-COVID-19 times, how have you changed your work routine to accommodate to the use of PPE?	reducing working hours	59	20	0	10	4	4	4	8	4	5	118	<0.010*
How has PPE affected your work efficiency?	more time being spent per patient, impaired vision, feeling of exhaustion/fatigue	81	14	12	8	0	4	4	6	6	2	137	<0.010*
How do you overcome difficulty in communication?	repeating loudly	35	2	9	12	3	0	4	10	6	3	84	<0.010*
	other means of communication	16	3	6	3	0	1	0	0	9	0	38	
What do you do to alleviate the patient's anxiety?	having a conversation and reassuring the patient	28	0	0	5	0	0	3	5	0	0	41	<0.010*
How do you explain the treatment plan and provide post-operative instructions to the patient?	on the dental chair while wearing PPE	126	25	21	13	1	16	4	3	10	2	231	<0.010*

Data presented as number (*n*). * statistically significant.

Discussion

This study assessed the common concerns among dentists about practicing during the COVID-19 pandemic as well as the overall effects of the pandemic on dental setups. Apart from its influence on clinical practice, the pandemic has also affected dental schools and dental university hospitals, resulting in partial or full closure. This in turn has had a negative impact on dental training and education, despite online teaching protocols.[5] Personal protective equipment prevents exposure to an infectious agent or a body fluid by creating a barrier between the potential infectious material and the health care worker.[6] It includes gloves, gowns, shoe covers, head covers, masks, respirators, face shields, and goggles. The responses indicate that 36% of the participants used gowns, masks/respirators, head covers/hoods, and face shields. It was found that 57% of the participants used an N95 mask, and 25% used both an N95 mask and a surgical mask for additional protection (Table 3). There are 2 issues to be considered with regard to the efficacy of a face mask – the filtration of the material and the fit of the design.[7] A surgical mask is a loose-fitting, disposable mask and does not provide complete protection. An N95/FFP2 respirator is designed to achieve a very close facial fit and very efficient filtration of airborne particles. The edges of the respirator are designed to form a seal around the nose and the mouth. The donning and doffing of PPE is a critical process that requires significant attention to detail. This process, particularly the removal and disposal of the contaminated PPE, is considered a very important step in limiting exposure to pathogens.[1]

When examining the changes introduced by the participants regarding their food intake pattern, 34% of the participants did not take any lunch/water breaks between patients and 33% preferred completing all of the patients for the day, and then removing their PPE. This could lead to ill effects, like dehydration, fatigue, irritability, and constant hunger, which could in turn affect their work. Eating more nutritious, immunity-boosting, vitamin-rich food was preferred by many, especially in this COVID-19 era. Some dentists skipped or delayed their meals because of an ongoing patient or to avoid the inconvenience of removing their PPE. This can prove harmful in the long run by causing serious side effects that include fatigue, skin problems, depression, and weight loss. According to this survey, 85% of the dentists reported that the arduous use of PPE affected their work efficiency. In fact, 35% of the participants said that they spent more time per patient owing to the use of PPE. Some endodontists responded that it took more time to complete a root canal, since their tactile sensation was diminished due to the use of double gloves. According to a survey conducted by Swaminathan et al.,

vision is impaired due to the presence of a face shield, and thus using a microscope has become difficult.[8] In the present study, it was found that the participants had a constant feeling of exhaustion due to the heat and perspiration caused by the use of PPE.

Dental professionals of all ages are experiencing increased levels of physical and emotional discomfort since resuming the routine care. The study found that 43% of the participants reported headaches, which is the 3rd most often reported side effect of implementing the advanced PPE protocols (Fig. 1). This could be a sign of dehydration.[9] To avoid or minimize headaches and dehydration, it is important to drink enough fluids during the day. Profuse sweating was reported by 74% of the respondents and 52% reported breathlessness. Taking short breaks throughout the day that allows one to remove their PPE may help overcome these difficulties. Reduced O_2 in the inspired air, CO_2 retention, rebreathing, and increased temperature, in isolation or combination, during prolonged PPE use could be a cause of physical exhaustion and breathlessness.[10] In the present survey, 28% of the respondents reported skin irritation or oily skin due to the use of face masks. The constant rubbing of the mask against the skin causes micro-tears, allowing easier entry for bacteria and dirt to clog the pores.[11] Exposure duration is considered to be the main risk factor for facial dermatitis, particularly when masks and goggles are worn for over 6 h. Washing hands more than 10 times a day may increase the risk of hand damage.[12]

The study findings support the authors' assumption that there is difficulty in communication due to the use of PPE (as reported by 89% of the participants). With the mouth being completely covered, safety glasses covering the eyebrows and face shields further muffling sound, in addition to the noisy working environment, both patients and the dental staff struggle to comprehend each other (as reported by 53% of the participants). Dentists have adapted themselves to overcome this communication gap. For example, 22% stated that repeating their instructions loudly was effective. However, in addition to frustration or miscommunication, raising one's voice for prolonged periods may lead to issues with voice strain and abuse.[12] A further 10% resorted to other means of communication, such as providing instructions via text messages or calls, writing on a piece of paper, or using a microphone with a small speaker. Non-verbal communication with hand gestures and other movements also proved to be effective.

Generally, when interacting with an unfamiliar face, people tend to focus their attention on the mouth and the eyes, as these are most expressive. For health care workers, it is difficult to convey a feeling of calmness or happiness, as almost 85% of the face is covered with PPE.[13] This can add to the stress and anxiety patients already feel owing to this health care crisis.[14] To alleviate this anxiety, the participants explained the importance of wearing PPE and how it provides safety to both the operator and the patient.[15] Having a conversation and reassuring the patient, or lightening the mood with a joke proved to be helpful. Communication is not just simply talking; the tone of the speech and expressions are important factors that influence it. Not only does the current use of masks and safety glasses make communication difficult, but it also causes digging on the bridge of the nose and the sides of the face, which may cause skin irritation. Prolonged side effects, like dehydration, sweating, fatigue, breathlessness, and headaches could be detrimental in the long run.

The use of PPE has been amplified during the COVID-19 pandemic; however, protection needs to be comfortable, not only to prevent fatigue or physical pain, but also for psychological well-being.[16]

Conclusions

Even though the use of PPE has become more important than ever, the results of this study show that it is a challenge for dentists in a clinical setup. As reported by the participants, there is difficulty in communication due to the added layers and the muffling of sound, and changes in work routines and food intake patterns may cause malnourishment and dehydration. There was a significant decrease in work efficiency owing to the added layers and their side effects. The most frequent side effect of PPE was profuse sweating, while the least experienced was a hoarse voice. All of the dentists faced side effects like dehydration, headaches, sweating, and fatigue, which could be detrimental in the long run.

In order to mitigate these problems, adapting to other means of communication, alleviating the patient's anxiety, taking regular breaks, drinking plenty of water, and having a healthy and balanced diet are essential for dentists in the COVID-19 era.

Ethics approval and consent to participate

The study was approved by the institutional Research Ethics Committee (No. of approval 144/IRB/YMTDC2021) after obtaining approval from 2 reviewers. The respondents participated in the study voluntarily.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Vallari Malandkar https://orcid.org/0000-0001-8417-5366
Sheetal Choudhari https://orcid.org/0000-0001-5798-3704
Dheeraj Kalra https://orcid.org/0000-0003-1220-7327
Parmeet Banga https://orcid.org/0000-0001-6769-9619

References

1. Ausmed. Donning and doffing PPE correctly. April 27, 2020. https://www.ausmed.com/cpd/articles/donning-doffing-ppe. Accessed May 17, 2021.
2. Gambarini G, Galli M, Gambarini E, et al. Fine aerosols and perceived risk of COVID-19 among Italian dental practitioners: An experimental survey. *J Contemp Dent Pract*. 2020;21(6):599–603. PMID:33025925.
3. Saatchi M, Abtahi M, Mohammadi G, Mirdamadi M, Binandeh ES. The prevalence of dental anxiety and fear in patients referred to Isfahan Dental School, Iran. *Dent Res J (Isfahan)*. 2015;12(3):248–253. PMID:26005465. PMCID:PMC4432608.
4. Kamp AA. Parent child separation during dental care: A survey of parent's preference. *Pediatr Dent*. 1992;14(4):231–235. PMID:1303521.
5. Gambarini G, Di Nardo D, Obino FV, et al. Academic dental activities and COVID-19 pandemic. *J Contemp Dent Pract*. 2020;21(6):598. PMID:33025924.
6. Allsopp J, Basu MK, Browne RM, Burge PS, Matthews JB. Survey of the use of personal protective equipment and prevalence of work related symptoms among dental staff. *Occup Environ Med*. 1997;54(2):125–134. doi:10.1136/oem.54.2.125
7. Howard J, Huang A, Li Z, et al. An evidence review of face masks against COVID-19. *Proc Natl Acad Sci U S A*. 2021;118(4):e2014564118. doi:10.1073/pnas.2014564118
8. Swaminathan R, Mukundadura BP, Prasad S. Impact of enhanced personal protective equipment on the physical and mental well-being of healthcare workers during COVID-19. *Postgrad Med J*. 2020. doi:10.1136/postgradmedj-2020-139150
9. Centers for Disease Control and Prevention (CDC). Using personal protective equipment (PPE). Updated August 19, 2020. https://www.cdc.gov/coronavirus/2019-ncov/hcp/using-ppe.html. Accessed April 30, 2021.
10. California Dental Association (CDA). Side effects of PPE: Tips to relieve physical and emotional exhaustion. August 17, 2020. https://www.cda.org/Home/News-and-Events/Newsroom/Article-Details/side-effects-of-ppe-tips-to-relieve-physical-and-emotional-exhaustion. Accessed May 7, 2021.
11. Davey SL, Lee BJ, Robbins T, Randeva H, Thake CD. Heat stress and PPE during COVID-19: Impact on healthcare workers' performance, safety and well-being in NHS settings. *J Hosp Infect*. 2021;108:185–188. doi:10.1016/j.jhin.2020.11.027
12. Hampton T, Crunkhorn R, Lowe N, et al. The negative impact of wearing personal protective equipment on communication during coronavirus disease 2019. *J Laryngol Otol*. 2020;134(7):577–581. doi:10.1017/S0022215120001437
13. Atzori L, Ferreli C, Atzori MG, Rongioletti F. COVID-19 and impact of personal protective equipment use: From occupational to generalized skin care need. *Dermatol Ther*. 2020;33(4):e13598. doi:10.1111/dth.13598
14. Jeddy N, Nithya S, Radhika T, Jeddy N. Dental anxiety and influencing factors: A cross-sectional questionnaire-based survey. *Indian J Dent Res*. 2018;29(1):10–15. doi:10.4103/ijdr.IJDR_33_17
15. Gadbury-Amyot CC, Williams KB, Overman PR, Glaros A. The effect of personal protective equipment on high- and low-dental fear patients. *J Dent Hyg*. 1994;68(2):75–81. PMID:7996212.
16. Ahmadi H, Ebrahimi A, Ghorbani F. The impact of COVID-19 pandemic on dental practice in Iran: A questionnaire-based report. *BMC Oral Health*. 2020;20(1):354. doi:10.1186/s12903-020-01341-x

Impact of the COVID-19 pandemic on the timing of dental care in Peruvian children

María Claudia Garcés-Elías[1,A–F], César Eduardo Del Castillo-López[1,A–F], Roberto Antonio León-Manco[1,A–F], Andrés Alonso Agudelo-Suárez[2,A–F]

[1] Faculty of Dentistry, Cayetano Heredia Peruvian University, Lima, Peru
[2] Faculty of Dentistry, University of Antioquia, Medellín, Colombia

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):37–44

Address for correspondence
María Claudia Garcés-Elías
E-mail: maria.garces@upch.pe

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on July 21, 2021
Reviewed on August 10, 2021
Accepted on August 23, 2021

Published online on February 15, 2022

Abstract

Background. Due to the high transmissibility of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), governments adopted preventive measures, such as social distancing and obligatory social immobilization, which negatively affected access to health services, including oral health services. Similarly, dental care restraint arose in this context, with the aim of reducing the possibility of cross-infection caused by aerosols, which notably restricted dental care activity.

Objectives. The aim of the study was to determine the impact of the COVID-19 pandemic on the timing of dental care in Peruvian children.

Material and methods. A cross-sectional study with a population of 42,115 respondents for 2019 and 20,510 for 2020 was conducted. The participants were children aged 0–11 years. The records of those who responded to the question on the time since their last dental care were considered, extracting a total of 22,166 (69.03%) subjects for 2019 and 9,945 (30.97%) subjects for 2020. The dependent variable consisted of the time since the last dental care measured in years; the variables of health, geographic and sociodemographic characteristics were grouped within 3 dimensions. Descriptive bivariate and multivariate analyses were applied by means of multiple linear regression in order to analyze the variables.

Results. The time since the last dental care during 2019 was 5.25 ±4.30 years, and it increased to 6.64 ±4.90 years in 2020. Within the multivariate analysis, the dimensions and their variables were ordered hierarchically for 2019 and 2020 separately, and as a whole. Each model was not significant when observed independently ($p > 0.05$); however, when evaluated as a whole, validity was observed only in model 1 of the year ($p = 0.018$), with $R^2 = 2.90$, a constant equal to 3.852, the non-standardized regression coefficient (β) of 1.653, and a 95% confidence interval (*CI*) of 0.289–3.018.

Conclusions. The 2020 COVID-19 pandemic year had a negative impact on the timing of dental care in Peruvian children, increasing it by 1.39 years as compared to 2019.

Keywords: delivery of health care, COVID-19, health service accessibility, dental care for children, cross-sectional studies

Cite as
Garcés-Elías MC, Del Castillo-López CE, León-Manco RA, Agudelo-Suárez AA. Impact of the COVID-19 pandemic on the timing of dental care in Peruvian children. *Dent Med Probl.* 2022;59(1):37–44. doi:10.17219/dmp/141521

DOI
10.17219/dmp/141521

Introduction

During its first months, the coronavirus disease 2019 (COVID-19) pandemic became the most complex socioeconomic problem of the last hundred years. It has disproportionately affected nations; Latin America has been one of the most affected regions during the period of the pandemic due to deficiencies in the infrastructure and response capacity of its health care systems, scarce investment in the health care systems by governments, and the pre-existing social disparities.[1,2] The risk of the spread of the virus led to the adoption of containment policies, such as social distancing and mandatory social immobilization, which had a negative influence on access to health services, without necessarily having a direct relationship with COVID-19. This problem also aggravated the health situation in vulnerable people and extreme age groups, especially in those with chronic conditions, reducing the possibility of timely diagnosis and treatment.[3] It is important to mention that the pandemic may have an adverse impact on health and medical care with regard to the acquisition of non-communicable diseases due to the lack of access to primary health services, especially in low- and middle-income countries.[4]

Taking into consideration dental services, it should be noted that it was recommended to delay or avoid visits, and treat only urgent cases in order to reduce the possibility of cross-infection caused by aerosols, which notably restricted dental care activity.[5,6] This was accompanied by high unemployment among oral health providers during the first months of the pandemic. However, practices and clinics were reopened with the incorporation of new biosafety standards, which in turn increased the cost of care. Even prior to the COVID-19 pandemic, there was an economic barrier in terms of access to oral health care. The new situation made it a major public health problem.[7]

A similar situation can be observed in Peru. As of March 12, 2020, several decrees were issued, such as the declaration of a national state of emergency,[8] the suspension of flights and the establishment of exceptional provisions to prevent the spread of the virus within the national territory.[9,10] Despite all the strategies adopted by the government, the community quarantine to counteract COVID-19 did not work in Peru. At the beginning of the quarantine, there were 71 confirmed cases, but by June 2020, when the quarantine ended, there were 268,602 confirmed cases and 8,761 deaths.[11] In addition, the negative impact of the pandemic on the health care system was evident, reflected in the degree of occupation of intensive care unit (ICU) beds at the national level, estimated at 93% of the total capacity, while some services in the interior of the country were already at their maximum capacity.[12] In order to make figures transparent, the government ordered a review of the clinical records of people who died due to the pandemic; consequently, the official figures were updated, showing a considerable increase from 69,342 to 185,380. In addition, this value allowed the identification of a mortality rate of 5,551 deaths due to COVID-19 per million inhabitants, which is the worst official figure worldwide.[13]

Meanwhile, in the field of national oral health care, there was a significant reduction in search for dental services; such behavior could be attributed to the suspension of certain economic activities, like dentistry, due to the fear of contagion as well as to following the provisions of immobility and the postponement of dental treatment. Highlighting that, despite a significant reduction in the number of patients, it was high time to reactivate dental services, as expressed in May 2020 in Supreme Decree No. 094-2020-PCM of the Peruvian government, which included dentistry among the activities allowed in new social coexistence.[14–16] It should be noted that subsequently, through Health Directive No. 100-MINSA-2020-DGIESP, the Peruvian Ministry of Health established provisions to guarantee the continuity of care during the pandemic by means of dental consultations in Peruvian health facilities.[17] The aim was to contribute to the alleviation of the impact of COVID-19 in terms of dental profession through an approach that promotes the reduction of infections, the establishment of biosecurity measures and the specifications of personal protective equipment (PPE), the ratification of disinfection and sterilization protocols, and the identification of risk factors for dental health care personnel. Consequently, the abovementioned recommendations were implemented nationwide, both in public and private dental setups. It was emphasized that during the pandemic, the provision of on-site dental services should be prioritized to emergency and urgent cases.[17] Likewise, in order to reduce the risk of mortality from infection, the criteria for scheduling immunization in the population over 18 years of age were established, prioritizing within 3 phases the individuals who work in the first line of defense, including the health sector workers, in order to preserve the integrity and continuity of the health care system.[18]

There are populations that face greater disparities in the use of health services, such as ethnic minorities, immigrants and those with low economic income, all of whom are more likely to lack dental insurance.[19] At the national level, the use of dental services among Peruvian children shows geographic and socioeconomic discrepancies; dental services are accessed mainly in the quintile with the highest economic capacity and residing in the capital of the country.[20,21] There is a clear need to produce more scientific evidence on access to dental care, evaluated from the perspective of the drastic changes caused by the pandemic; in this sense, the purpose of this research was to determine the impact of the COVID-19 pandemic on the timing of dental care in Peruvian children.

Material and methods

This research used the databases of the 2019 and 2020 Demographic and Family Health Surveys (ENDES), developed by the National Institute of Statistics and Informatics of Peru (INEI). The ENDES is a survey applied annually through home interviews that has a stratified 2-stage cluster-sample design, representative at national, regional, urban, and rural levels. Additionally, it provides information on access to dental care in children aged 0–11 years. In 2019, a sample size of 36,760 dwellings was considered, with a total of 42,115 respondents, while in 2020, the sample size was 37,390 dwellings and 20,510 respondents. A decrease in the number of participants between the 2 years may be due to the COVID-19 pandemic. For the present study, only the records of those subjects who responded to the question about the time since their last dental care were considered, extracting a total of 32,111, with 22,166 (69.03%) subjects for 2019 and 9,945 (30.97%) subjects for 2020. For both years, the surveys were applied from January to December; however, the 2020 survey involved changes due to the health emergency situation and the need to reach the established sample, which led to the implementation of telephone interviews and the subsequent return to face-to-face interviews, maintaining the biosecurity of the field personnel.[22]

Regarding the definition of variables, the time since the last dental care measured in years was considered the dependent variable. For the independent variable, the year was categorized as 2019 and 2020, considering the latter as the period in which the COVID-19 pandemic developed in Peru. In addition, other covariates were incorporated into the analysis, which were grouped into 3 dimensions, namely health characteristics, geographic characteristics and sociodemographic characteristics. The first of these included the possession of health insurance and the place of dental care, categorized into care provided by the Ministry of Health, Social Security (ESSALUD), Armed Forces and Police (FFAA/PNP), and the private sector. With respect to geographic characteristics, the area of residence was categorized as urban, rural or natural region, which could be defined as the Lima metropolitan area (the capital of the country), the rest of the coast, highlands, and jungle. The place of residence was classified as capital, city, town, and countryside. Finally, altitude was dichotomized into less than 2,500 meters above mean sea level (MAMSL) and equal to or more than 2,500 MAMSL. Sociodemographic characteristics were catalogued in quintiles of wealth, a variable composed of the particularities and disposition to some consumption of goods and services that each household possesses. Later, through the methodology applied in the U.S. Demographic and Health Surveys Program,[23] a score was assigned to each household and the same score was assigned to each one of its residents, which allowed stratifying each household from the 1st quintile (the poorest) to the 5th quintile (the richest).[23,24] Age was grouped as 0–5 years and 6–11 years. Finally, the sex variable was considered. It is important to mention that these covariates were also analyzed in previous studies.[20,25,26]

Statistical analysis

The ENDES databases were extracted from the official INEI website (http://iinei.inei.gob.pe/microdatos/) through various modules, which were unified into a single database for the subsequent analysis with the use of the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). The analysis was carried out using the complex samples module, since ENDES is a national survey with possible representative estimates.

A descriptive analysis of the qualitative variables was carried out by means of absolute and relative frequencies. For the variable time since the last dental care, the mean and standard deviation ($M \pm SD$) values were obtained. In addition, the normality of the distribution of the dependent variable was evaluated according to other variables with the Kolmogorov–Smirnov test. Subsequently, nonparametric tests were applied – the Mann–Whitney U test for dichotomous variables and the Kruskal–Wallis test for polytomous variables. Next, hierarchical multiple linear regression was developed with the purpose of generating models between the independent variables and the time since the last dental care, according to the dimensions to be analyzed by years separately and together. It is important to mention that a logarithmic transformation was previously applied to the dependent variable due to the lack of normal distribution. The confidence level in the study was 95%, and $p < 0.05$ was considered an indicator of statistical significance in all tests.

Results

The time since the last dental care in 2019 was 5.25 ±4.30 years, and it increased to 6.64 ±4.90 years in 2020. This difference was statistically significant ($p < 0.001$) and represented an increase of 1.39 years. In 2019, the time since the last dental care differed significantly with regard to health insurance tenure, the area of residence, the natural region of residency, altitude, age, and sex ($p < 0.05$). In 2020, differences regarded the place of dental care and the natural region of residency ($p < 0.05$) (Table 1).

A hierarchical multiple linear regression analysis was also performed, considering the models in years

Table 1. Year, health, geographic and sociodemographic characteristics with reference to the time since the last dental care in Peruvian children in 2019 and 2020

Variables			Total *n* (%)	2019			2020		
				n (%)	time since the last dental care [years] *M* ±*SD*	*p*-value	*n* (%)	time since the last dental care [years] *M* ±*SD*	*p*-value
Year of the COVID-19 pandemic *p* <0.001†*			32,111 (100.00)	22,166 (69.30)	5.25 ±4.30	–	9,945 (30.97)	6.64 ±4.90	–
Health characteristics	health insurance	yes	26,008 (80.99)	17,874 (80.64)	5.19 ±4.29	<0.001†*	8,134 (81.79)	6.62 ±4.89	0.475†
		no	6,103 (19.01)	4,292 (19.36)	5.48 ±4.32		1,811 (18.21)	6.73 ±4.97	
	place of dental care *n* = 31,990 (22,070/9,920)	Ministry of Health	19,315 (60.38)	13,479 (61.07)	5.23 ±4.28	0.155‡	5,836 (58.83)	6.76 ±4.89	<0.001‡*
		Social Security	4,990 (15.60)	3,486 (15.80)	5.34 ±4.36		1,504 (15.16)	7.30 ±5.06	
		Armed Forces and Police	106 (0.33)	89 (0.40)	4.36 ±3.83		17 (0.17)	8.94 ±5.08	
		private sector	7,579 (23.69)	5,016 (22.73)	5.24 ±4.31		2,563 (25.84)	5.96 ±4.83	
Geographic characteristics *n* = 21,434 (14,740/6,694)	area of residence	urban	15,558 (75.29)	10,631 (72.12)	5.41 ±4.35	0.006†*	4,927 (73.60)	6.66 ±4.87	0.790
		rural	5,876 (27.41)	4,109 (27.88)	5.19 ±4.26		1,767 (26.40)	6.66 ±4.86	
	natural region of residency	Lima metropolitan area	2,983 (13.92)	1,944 (13.19)	5.46 ±4.45	<0.001‡*	1,039 (15.52)	7.04 ±5.13	<0.001‡*
		rest of the coast	6,335 (29.56)	4,350 (29.51)	5.49 ±4.35		1,985 (29.65)	6.81±4.81	
		highlands	7,570 (35.32)	5,324 (36.12)	5.15 ±4.28		2,246 (33.55)	6.68 ±4.89	
		jungle	4,546 (21.21)	3,122 (21.18)	5.43 ±4.28		1,424 (21.27)	6.12 ±4.67	
	place of residence	capital	2,983 (13.92)	1,944 (13.19)	5.46 ±4.45	0.054‡	1,039 (15.52)	7.04 ±5.13	0.140‡
		city	6,599 (30.79)	4,509 (30.59)	5.41 ±4.39		2,090 (31.22)	6.52 ±4.79	
		town	5,976 (27.88)	4,178 (28.34)	5.39 ±4.27		1,798 (26.86)	6.59 ±4.81	
		countryside	5,876 (27.41)	4,109 (27.88)	5.19 ±4.26		1,767 (26.40)	6.66 ±4.86	
	altitude	<2,500 MAMSL	14,988 (69.93)	10,215 (69.30)	5.43 ±4.35	<0.001†*	4,773 (71.30)	6.62 ±4.88	0.300†
		>2,500 MAMSL	6,446 (30.07)	4,525 (30.70)	5.17 ±4.27		1,921 (28.70)	6.75 ±4.85	
Sociodemographic characteristics	quintile of wealth *n* = 20,381 (14,019/6,362)	1st quintile	4,689 (23.01)	3,326 (23.72)	5.27 ±4.27	0.567‡	1,363 (21.42)	6.59 ±4.86	0.794‡
		2nd quintile	5,336 (28.18)	3,748 (26.74)	5.36 ±4.26		1,588 (24.96)	6.71 ±4.76	
		3rd quintile	4,358 (21.38)	2,936 (20.94)	5.46 ±4.42		1,422 (22.35)	6.70 ±4.90	
		4th quintile	3,423 (16.80)	2,327 (16.60)	5.35 ±4.33		1,096 (17.23)	6.72 ±4.95	
		5th quintile	2,575 (12.63)	1,682 (12.00)	5.30 ±4.39		893 (14.04)	6.63 ±5.05	
	age [years]	0–5	14,607 (45.49)	10,237 (46.18)	4.95 ±4.29	<0.001†*	4,370 (43.94)	6.64 ±5.05	0.068†
		6–11	17,504 (54.51)	11,929 (53.82)	5.51 ±4.29		5,575 (56.06)	6.65 ±4.79	
	sex	male	18,643 (58.06)	12,860 (58.02)	5.31 ±4.32	0.019†*	5,783 (58.15)	6.65 ±4.90	0.778†
		female	13,468 (41.94)	9,306 (41.98)	5.17 ±4.28		4,162 (41.85)	6.63 ±4.91	

n – number; *M* – mean; *SD* – standard deviation; COVID-19 – coronavirus disease 2019; MAMSL – meters above mean sea level; † Mann–Whitney U test; ‡ Kruskal–Wallis test; * statistically significant.

separately and as a whole. When the analysis was done by separate years, the models were not statistically significantly different for any variable ($p > 0.05$) (Table 2); however, when analyzed as a whole and taking into account the year as a variable, only model 1 of the year was valid ($p = 0.018$), with $R^2 = 2.90$, a constant equal to 3.852, the non-standardized regression coefficient (β) of 1.653, and a 95% confidence interval (*CI*) of 0.289–3.018. Although other models were not significant, the statistical significance of the year variable was maintained in all of them ($p < 0.05$) (Table 3).

Discussion

Due to the COVID-19 pandemic, inequalities in terms of equitable health care have increased, disadvantaging certain minority communities that face barriers in access to health services.[27] This situation may have indirect detrimental effects, such as an increase in morbidities due to the lack of preventive services, untimely diagnoses and the cessation of treatment for chronic pathologies, impacting more significantly fragile economies, like those of developing countries.[28]

Table 2. Hierarchical multiple regression models for health, geographic and sociodemographic characteristics with reference to the time since the last dental care in the study sample ($N = 32,111$)

Variables				R^2 [%]	Change of R^2	*p*-value (change of R^2)	Constant	Non-standardized regression coefficient	Standardized regression coefficient	95% *CI*	*p*-value	*p*-value (model)
2019	model 1	health	health insurance	0.20	0.20	0.885	5.880	–0.311	–0.028	–2.155 to 1.534	0.740	0.885
			place of dental care					0.113	0.031	–0.498 to 0.724	0.715	
	model 2	health	health insurance	3.20	3.00	0.382	6.995	1.821	0.148	–1.606 to 5.247	0.290	0.616
			place of dental care					0.009	0.002	–1.131 to 1.148	0.998	
		geographic	area of residence					–1.731	–0.146	–4.677 to 1.216	0.247	
			natural region of residency					0.080	0.019	–0.758 to 0.918	0.851	
			place of residence					0.808	0.183	–0.361 to 1.978	0.174	
			altitude					–0.970	–0.099	–2.829 to 0.888	0.304	
	model 3	health	health insurance	6.40	3.20	0.492	8.818	–0.413	–0.038	–2.346 to 1.520	0.641	0.633
			place of dental care					0.158	0.044	–0.512 to 0.829	0.641	
		geographic	area of residency					–1.953	–0.165	–5.188 to 1.282	0.234	
			natural region of residency					0.179	0.044	–0.721 to 1.079	0.695	
			place of residence					0.692	0.157	–0.535 to 1.919	0.266	
			altitude					–1.427	–0.146	–3.387 to 0.534	0.152	
		socio-demographic	quintile of wealth					0.295	0.095	–0.510 to 1.100	0.470	
			age					–4.869	–0.101	–13.289 to 3.497	0.251	
			sex					–0.091	–0.011	–1.580 to 1.398	0.904	
2020	model 1	health	health insurance	2.20	2.20	0.571	3.746	1.803	0.147	–1.649 to 5.254	0.299	0.571
			place of dental care					0.083	0.021	–1.050 to 1.216	0.883	
	model 2	health	health insurance	12.70	10.40	0.258	9.213	1.821	0.148	–1.606 to 5.247	0.290	0.371
			place of dental care					0.009	0.002	–1.131 to 1.148	0.998	
		geographic	area of residence					–2.656	–0.187	–9.044 to 3.732	0.407	
			natural region of residency					–1.178	–0.242	–3.037 to 0.680	0.208	
			place of residence					–0.011	–0.002	–2.624 to 2.603	0.993	
			altitude					0.319	0.029	–3.380 to 4.018	0.863	
	model 3	health	health insurance	20.00	7.40	0.435	7.957	1.451	0.118	–2.127 to 5.028	0.418	0.420
			place of dental care					–0.160	–0.040	–1.366 to 1.046	0.790	
		geographic	area of residence					–4.511	–0.318	–11.813 to 2.791	0.219	
			natural region of residency					–1.609	–0.330	–3.679 to 0.460	0.124	
			place of residence					0.511	0.103	–2.226 to 3.248	0.708	
			altitude					–0.505	–0.045	–4.328 to 3.319	0.791	
		socio-demographic	quintile of wealth					–0.746	–0.195	–2.499 to 1.006	0.395	
			age					–5.130	–0.980	–13.181 to 3.245	0.224	
			sex					1.253	0.125	–1.699 to 4.206	0.393	

R^2 – determination coefficient; *CI* – confidence interval.

This study reveals an increase of 1 year in the time since the last dental care as compared to the year prior to the outbreak of the COVID-19 pandemic in children under 12 years of age. Previous research by Azañedo et al.,[29] Hernández-Vásquez et al.,[21] and Aravena-Rivas and Carbajal-Rodríguez[20] identified certain determinants of access to oral health services, such as the natural region of residency, living in an urban or rural area, the wealth quintile, the educational level of caregivers, and age. It was observed that the Peruvian population under 12 years of age had limited access to dental services.[20,21,29]

Table 3. Hierarchical multiple regression models for year, health, geographic and sociodemographic characteristics with reference to the time since the last dental care in the study sample (N = 32,111)

Variables			R^2 [%]	Change of R^2	p-value (change of R^2)	Constant	Non-standardized regression coefficient	Standardized regression coefficient	95% CI	p-value	p-value (model)
Model 1	year of the COVID-19 pandemic		2.90	2.90	0.018*	3.852	1.653	0.170	0.289 to 3.018	0.018*	0.018*
Model 2	year of the COVID-19 pandemic		3.00	0.20	0.858	3.080	1.657	0.170	0.286 to 3.029	0.018*	0.117
	health	health insurance					0.315	0.027	−1.311 to 1.942	0.703	
		place of dental care					0.109	0.028	−0.429 to 0.646	0.691	
Model 3	year of the COVID-19 pandemic		5.20	2.20	0.378	5.584	1.755	0.180	0.364 to 3.146	0.014*	0.184
	health	health insurance					0.278	0.024	−1.383 to 1.938	0.742	
		place of dental care					0.050	0.013	−4.970 to 0.596	0.858	
	geographic	area of residence					−1.925	−0.151	−4.618 to 0.769	0.160	
		natural region of residency					−0.208	−0.048	−0.980 to 0.563	0.595	
		place of residence					0.470	0.102	−604 to 1.544	0.389	
		altitude					−0.718	−0.069	−2.381 to 0.946	0.396	
Model 4	year of the COVID-19 pandemic		8.40	3.10	0.291	9.522	1.922	0.198	0.487 to 3.357	0.009*	0.182
	health	health insurance					0.148	0.013	−1.538 to 1.834	0.862	
		place of dental care					0.111	0.029	−0.456 to 0.677	0.700	
	geographic	area of residence					−2.732	−0.215	−5.681 to 0.216	0.069	
		natural region of residency					−0.269	−0.061	−1.093 to 0.556	0.521	
		place of residence					0.395	0.086	−0.707 to 1.497	0.480	
		altitude					−1.101	−0.106	−2.814 to 0.611	0.206	
	socio-demographic	quintile of wealth					−0.048	−0.015	−0.762 to 0.666	0.894	
		age					−5.690	−0.094	−14.372 to 2.991	0.198	
		sex					0.285	0.031	−1.035 to 1.606	0.670	

* statistically significant.

It is important to consider that during 2019 there were significant differences in the timing of dental care according to health insurance tenure, the area of residence, the natural region of residency, altitude, age, and sex. Understanding that, under normal conditions, i.e., without the COVID-19 pandemic, these characteristics determined disparities in relation to the time it took for an individual to be attended. However, for 2020, there were modifications in factors that produced these differences in previous years, as is the case of the place of care, where the contribution of the Ministry of Health, Armed Forces and Police facilities considerably increased the time since the last dental care. Private health care institutions, however, did not present major changes in this aspect as compared to a previous period. Regarding the natural region of origin, there was a generalized increase in temporality, which would indicate that persons under 12 years of age stopped receiving care at the national level.

Although the results show an increase in all of the variables studied, it can be said that there are no longer significant differences between them. In addition, it is understood that of all the characteristics evaluated in the year 2020, the only one that shows great relevance is the year of analysis, which presents significant differences as a variable and as an explanatory model in itself. In this sense, the year had a negative impact on the timing of dental care in Peruvian children under 12 years of age. Also, some other studies mention access to dental care during the pandemic. Firstly, Brian and Weintraub stated that accessibility had been limited, especially in populations with a higher risk of COVID-19, and they also emphasized the measures that were advised, such as avoiding dental care unless it was an emergency, and in cases of COVID-19, delaying it until recovery.[30] Likewise, Kranz et al. reported that 74.7% of adults delayed their dental visits for any type of preventive or recuperative care due to the pandemic.[31]

In accordance with the findings of the present study, it is important to mention that multiple legal provisions and strategies to contain the spread of the virus and strengthen the quarantine were implemented in Peru[8–10]; however, these did not produce the expected results, with a disproportionate increase in both the number of infections and the number of deaths.[11] In addition, the national health care system faced one of the most critical scenarios ever experienced, such as the deficit of ICU beds. The correction of the underreporting of positive cases and deaths

provided hopeless figures that showed the magnitude and severity of the problem, as it translated into the worst global case fatality rate.[12,13] From a dental perspective, the pandemic led to the abrupt interruption of activities, which were allowed and resumed much later within the context of new normality, through policies that ratified biosecurity and the prioritization of care for emergency and urgent cases.[14–17] Subsequently, with the aim of protecting and strengthening the health care human resources, the program of immunization against COVID-19 was initiated, benefiting the health care personnel, including dentists.[18]

Limitations

As for the limitations in the development of this research, its cross-sectional design stands out, which makes it impossible to infer causal relationships with regard to the results.

Furthermore, secondary data from ENDES was used, and there could be a possible emergence of memory bias due to the self-reported information provided by the respondents as well as the loss of information for data analysis.

Finally, it is important to consider that in previous years there were already circumstances that complicated and delayed access to dental care in the country; however, the drastic changes resulting from the COVID-19 pandemic had a preponderant influence on all health care systems and multiple sectors that make up society, with negative repercussions on the accessibility of dental care, either because of the measures implemented by the government or because of the consequences they brought with them. It is evident that within the context of COVID-19, both personal habits and health services underwent important modifications to control the number of infections; however, a radical change in the timing of dental care suggests that the pre-existing problems with access to dental care have increased, especially in vulnerable populations, such as children, even though the state has implemented and strengthened contingency strategies, like teledentistry.

Conclusions

The 2020 COVID-19 pandemic year negatively impacted the timing of dental care in Peruvian children, increasing it by 1.39 years as compared to 2019.

Ethics approval and consent to participate

The study was developed with the open access information provided by the National Institute of Statistics and Informatics of Peru (INEI). Therefore, no ethical approval was required, with regard to the secondary analysis of anonymous information about the study subjects.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

María Claudia Garcés-Elías https://orcid.org/0000-0003-4873-7661
César Eduardo Del Castillo-López https://orcid.org/0000-0001-6831-897X
Roberto Antonio León-Manco https://orcid.org/0000-0001-9641-1047
Andrés Alonso Agudelo-Suárez https://orcid.org/0000-0002-8079-807X

References

1. Bański J, Mazur M, Kamińska W. Socioeconomic conditioning of the development of the COVID-19 pandemic and its global spatial differentiation. *Int J Environ Res Public Health*. 2021;18(9):4802. doi:10.3390/ijerph18094802
2. Santander-Gordon D, Iturralde GA, Freire-Paspuel B, et al. Crucial contribution of the universities to SARS-CoV-2 surveillance in Ecuador: Lessons for developing countries. *One Health*. 2021;13:100267. doi:10.1016/j.onehlt.2021.100267
3. Bambra C, Riordan R, Ford J, Matthews F. The COVID-19 pandemic and health inequalities. *J Epidemiol Community Health*. 2020;74(11):964–968. doi:10.1136/jech-2020-214401
4. Bullen C, McCormack J, Calder A, et al. The impact of COVID-19 on the care of people living with noncommunicable diseases in low- and middle-income countries: An online survey of physicians and pharmacists in nine countries. *Prim Health Care Res Dev*. 2021;22:e30. doi:10.1017/S146342362100030X
5. Nair AK, Mathew P, Sreela LS, Prasad TS, Jose M. Knowledge and attitude toward COVID-19 and dental treatment – its availability and treatment satisfaction during the pandemic among adult population – an online survey. *J Educ Health Promot*. 2021;10:77. doi:10.4103/jehp.jehp_800_20
6. Lundberg A, Hillebrecht AL, McKenna G, Srinivasan M. COVID-19: Impacts on oral healthcare delivery in dependent older adults. *Gerodontology*. 2021;38(2):174–178. doi:10.1111/ger.12509
7. Elster N, Parsi K. Oral health matters: The ethics of providing oral health during COVID-19. *HEC Forum*. 2021;33(1–2):157–164. doi:10.1007/s10730-020-09435-3
8. Government of Peru, Ministry of Health. DS No. 008-2020-SA. Decreto Supremo que declara en Emergencia Sanitaria a nivel nacional por el plazo de noventa (90) días calendario y dicta medidas de prevención y control del COVID-19. Lima, Peru: Government of Peru; 2020. https://cdn.www.gob.pe/uploads/document/file/605928/DS_008-2020-SA.PDF. Accessed June 15, 2021.
9. Government of Peru, Ministry of Transport and Communication. DS No. 008-2020-MTC. Suspenden los vuelos provenientes de Europa y Asia, y desde el territorio nacional hacia dichos destinos, por el plazo de treinta (30) días calendarios a partir del 16 de marzo de 2020. Lima, Peru: Government of Peru; 2020. https://cdn.www.gob.pe/uploads/document/file/565943/DS_008-2020-MTC.pdf. Accessed June 17, 2021.
10. Government of Peru. DS No. 044-2020-PCM. Decreto Supremo que declara Estado de Emergencia Nacional por las graves circunstancias que afectan la vida de la Nación a consecuencia del brote del COVID-19. Lima, Peru: Government of Peru; 2020. https://cdn.www.gob.pe/uploads/document/file/566448/DS044-PCM_1864948-2.pdf. Accessed June 17, 2021.
11. Lossio J. Covid-19 in Peru: State and social responses [in Spanish]. *Hist Cienc Saude Manguinhos*. 2021;28(2):581–585. doi:10.1590/S0104-59702021005000001

12. Benítez MA, Velasco C, Sequeira AR, Henríquez J, Menezes FM, Paolucci F. Responses to COVID-19 in five Latin American countries. *Health Policy Technol*. 2020;9(4):525–559. doi:10.1016/j.hlpt.2020.08.014
13. Dyer O. Covid-19: Peru's official death toll triples to become world's highest. *BMJ*. 2021;373:n1442. doi:10.1136/bmj.n1442
14. Guo H, Zhou Y, Liu X, Tan J. The impact of the COVID-19 epidemic on the utilization of emergency dental services. *J Dent Sci*. 2020;15(4):564–567. doi:10.1016/j.jds.2020.02.002
15. Xu S, Li Y. Beware of the second wave of COVID-19. *Lancet*. 2020;395(10233):1321–1322. doi:10.1016/S0140-6736(20)30845-X
16. Government of Peru. DS No. 094-2020-PCM. Decreto Supremo que establece las medidas que debe observar la ciudadanía hacia una nueva convivencia y prórroga el Estado de Emergencia Nacional por las graves ciurcunstancias que afectan la vida de la Nación a consecuencia del COVID-19. Lima, Peru: Government of Peru; 2020. https://cdn.www.gob.pe/uploads/document/file/730522/DS_N__094-2020-PCM.pdf. Accessed June 17, 2021.
17. Government of Peru. Directiva Sanitaria No. 100/MINSA/2020/DGIESP. Manejo de la atención estomatológica en el contexto de la pandemia por COVID-19. Lima, Peru: Government of Peru; 2020. https://cdn.www.gob.pe/uploads/document/file/716209/DIRECTIVA_SANITARIA_N_100-MINSA-2020-DGIESP.pdf. Accessed June 20, 2021.
18. Government of Peru. Directiva Sanitaria No. 129-MINSA/2021/DGIESP. Directiva sanitaria para la vacunación contra la COVID-19 en la situación de emergencia sanitaria por la pandemia en el Perú. Lima, Peru: Government of Peru; 2021. https://cdn.www.gob.pe/uploads/document/file/1645872/Directiva%20Sanitaria%20N%C2%BA129-MINSA/2021/DGIESP.pdf. Accessed June 20, 2021.
19. Kalash DA. How COVID-19 deepens child oral health inequities. *J Am Dent Assoc*. 2020;151(9):643–645. doi:10.1016/j.adaj.2020.05.015
20. Aravena-Rivas Y, Carbajal-Rodríguez G. Geographical and socioeconomic inequalities in dental attendance among children in Peru: Findings from the Demographic and Family Health Survey 2017. *Community Dent Oral Epidemiol*. 2021;49(1):78–86. doi:10.1111/cdoe.12580
21. Hernández-Vásquez A, Azañedo D, Díaz-Seijas D, et al. Access to dental health services in children under twelve years of age in Peru, 2014 [in Spanish]. *Salud Colect*. 2016;12(3):429–441. doi:10.18294/sc.2016.912
22. National Institute of Statistics and Informatics, Peru. Demographic and Family Health Survey. 2021. https://proyectos.inei.gob.pe/endes/. Accessed June 28, 2021.
23. United States Agency for International Development. The Demographic and Health Surveys Program. Wealth index. https://dhsprogram.com/topics/wealth-index/. Accessed July 19, 2021.
24. National Institute of Statistics and Informatics, Peru. Demographic and Family Health Survey. 2014. https://www.inei.gob.pe/media/MenuRecursivo/publicaciones_digitales/Est/Lib1153/libro.pdf. Accessed July 19, 2021.
25. Carbajal-Rodriguez G, Aravena-Rivas Y, León-Manco R. Social gradients in dental attendance among older adults: Findings from the National Survey on Demographics and Health in Peru. *Community Dent Health*. 2019;36(4):281–286. doi:10.1922/CDH_4597Carbajal-Rodriguez06
26. Lang IA, Gibbs SJ, Steel N, Melzer D. Neighbourhood deprivation and dental service use: A cross-sectional analysis of older people in England. *J Public Health (Oxf)*. 2008;30(4):472–478. doi:10.1093/pubmed/fdn047
27. Farquharson WH, Thornton CJ. Debate: Exposing the most serious infirmity – racism's impact on health in the era of COVID-19. *Child Adolesc Ment Health*. 2020;25(3):182–183. doi:10.1111/camh.12407
28. Roy CM, Bollman EB, Carson LM, Northrop AJ, Jackson EF, Moresky RT. Assessing the indirect effects of COVID-19 on healthcare delivery, utilization and health outcomes: A scoping review. *Eur J Public Health*. 2021;31(3):634–640. doi:10.1093/eurpub/ckab047
29. Azañedo D, Hernández-Vásquez A, Casas-Bendezú M, Gutiérrez C, Agudelo-Suárez AA, Cortés S. Factors determining access to oral health services among children aged less than 12 years in Peru. *F1000Res*. 2017;6:1680. doi:10.12688/f1000research.12474.1
30. Brian Z, Weintraub JA. Oral health and COVID-19: Increasing the need for prevention and access. *Prev Chronic Dis*. 2020;17:E82. doi:10.5888/pcd17.200266
31. Kranz AM, Gahlon G, Dick AW, Stein BD. Characteristics of US adults delaying dental care due to the COVID-19 pandemic. *JDR Clin Trans Res*. 2021;6(1):8–14. doi:10.1177/2380084420962778

Duration and dose of chemotherapy and dental development

Anna Jodłowska[A–D], Lidia Postek-Stefańska[E,F]

Department of Pediatric Dentistry, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):45–58

Address for correspondence
Anna Jodłowska
E-mail: wega25@poczta.onet.pl

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on March 30, 2021
Reviewed on June 3, 2021
Accepted on June 14, 2021

Published online on March 31, 2022

Abstract

Background. Given the susceptibility of developing tissues to drugs, even small doses of anticancer drugs may affect odontogenesis. Although any toxic effect is transient, the treatment regimens are based on repeated drug administration.

Objectives. The study aimed to establish the impact of antineoplastic therapy on the occurrence of long-term adverse dental effects in a dose-dependent manner in young survivors treated for cancer before 10 years of age.

Material and methods. In total, 37 cancer survivors treated with antineoplastic therapy before 10 years of age underwent a dental examination with a thorough analysis of panoramic radiographs. A total of 236 teeth with 243 different developmental abnormalities were revealed in 28 survivors. Agenesis, tooth size reduction, taurodontia, and enamel and root abnormalities were diagnosed. All survivors received multi-agent chemotherapy, with the most frequently used drugs being vincristine (VCR), doxorubicin (DXR), cyclophosphamide (CP), ifosfamide (IF), etoposide (VP-16), carboplatin (CBDCA), cisplatin (CDDP), and actinomycin-D (ActD). A detailed analysis of medical records was also performed to assess the relationship between the treatment duration as well as the cumulative drug dose administered and the occurrence of particular disturbances.

Results. When analyzing the treatment duration and the drug doses in the affected and non-affected participants, there were no statistically significant differences between the survivors with different disturbances within most of the specific drug groups. In some groups, the mean cumulative treatment dose was significantly higher in the non-affected patients. According to Spearman's rho, no significant relationships were observed.

Conclusions. In the present study, no significant differences in terms of treatment duration or drug doses were observed between the patients with particular abnormalities. The developmental stage of tooth formation during chemotherapy is likely the most important factor influencing dental changes. For future research with respect to different treatment protocols, an analysis of a more homogenous group of survivors is warranted.

Keywords: tooth abnormalities, dental development, chemotherapy

Cite as
Jodłowska A, Postek-Stefańska L. Duration and dose of chemotherapy and dental development. *Dent Med Probl.* 2022;59(1):45–58. doi:10.17219/dmp/138914

DOI
10.17219/dmp/138914

Introduction

Anticancer treatment in children is typically very effective. However, the treatment can result in acute and late adverse effects, including effects on developing dental structures. Various standard treatment models have been established for the management of malignant diseases. Some antineoplastic drugs, such as vincristine (VCR), etoposide (VP-16) and cyclophosphamide (CP), are used relatively often in the treatment regimens for different cancers. Some of these drugs are dedicated to specific cancer types. However, they all have a low therapeutic index, and, through different mechanisms of action, ultimately result in arrested cell division or apoptosis. Vincristine and alternative microtubule-binding vinca alkaloids still remain the first choice of cytostatic agent for the therapy of solid tumors and blood cancers. They cause the cessation of cellular division in the M phase, followed by cell apoptosis.[1] Doxorubicin (DXR) stabilizes the complexes of double-stranded DNA and topoisomerase IIα, which helps the enzyme cut both DNA strands.[2] Cyclophosphamide and similar alkylating drugs, such as ifosfamide (IF), cross-link guanine bases in DNA.[3] Actinomycin D (ActD), like other antibiotics, self-inserts into DNA and causes damage to its structure, resulting in the inhibition of RNA polymerase and protein synthesis.[4] Cisplatin (CDDP) and carboplatin (CBDCA) represent a group of platinum-based cytotoxic drugs widely administered as part of therapy for various solid tumors. Their initial interaction with purine bases in DNA eventually results in cellular death.[5] Etoposide (VP-16) destroys the double-stranded structure of DNA by inhibiting type II topoisomerase and is used in the treatment protocols for many different cancers.[6]

Variability in treatment options and, for the same reason, possible differences in cytotoxic impact may be the deciding factors for the occurrence of long-term effects. However, the multi-drug nature of anticancer therapy makes it difficult to estimate the possible toxic impact of particular medications on odontogenesis. Considering the similarity of all developing tissues, we can surmise that the rapidly dividing dental cells are highly susceptible to toxic damage in the same manner as it is observed in cancer. Therefore, even a small dose of a drug may cause irreversible changes in odontogenesis. However, since the half-life of chemotherapeutic agents is short, their toxic effects could be transient, also in the case of fully developed cells.[7]

As far as developing dental tissues are concerned, chemotherapy-induced changes may be observed in either the rapidly dividing immature cells or fully developed hard tissue-forming cells. This has been well documented in vitro and in animal studies.[1,3] It has been reported that vinblastine (VBL) causes the dose-dependent inhibition of dentinogenesis in rat incisors.[8] Cyclophosphamide has been shown to inhibit cell division in the immature portion of the rat tooth germ, and to cause mutations in more developed dentin and enamel precursor cells when administered at low or high doses, respectively.[3] The treatment dose is likely to be the main factor influencing dental abnormalities. Another experimental study tested the effects of VCR on rat incisors.[9] The authors reported that after toxic drug administration, the observed mitotic cessation of young cell division, as well as changes in the secretory activity of mature odontoblasts were transient. The disturbances of odontogenesis occurred locally and were followed by the correct matrix secretion.[9] However, intensive chemotherapeutic regimens are based on repeated drug administration at standard treatment intervals. Therefore, a developing tooth germ is exposed to the regular toxic impact of multi-drug therapy. Repeated treatment may be another factor responsible for the dental sequelae of chemotherapy.

In the literature, dental disturbances induced by antineoplastic treatment are reported in approx. 70% of cancer survivors (62.29–71%).[10–13] The peak incidence of affected teeth has been reported in survivors who received chemotherapy at a young age.[3,7,11,14–19] Nevertheless, attempts to estimate the effects of individual drugs or their doses on human dental development are on-going.[12,20–22]

The present study aimed to establish the impact of antineoplastic therapy together with any dose-dependent relationship on the occurrence of long-term adverse dental effects in survivors treated for cancer before 10 years of age.

Material and methods

The study was approved by the Bioethics Committee at the Medical University of Silesia, Katowice, Poland, on February 25, 2013 and November 29, 2016 (KNW/0022/KB1/15/I/13 and KNW/0022/KB1/15/II/16, respectively). Dental examinations were carried out in cancer survivors who fulfilled the following inclusion criteria: anticancer therapy started before 10 years of age and was completed at least 2 years before the dental examination. The caregivers of 37 individuals aged 6–17 years provided informed written consent for dental examinations, including panoramic radiographs, and the processing of medical data concerning the details of anticancer treatment. In the examined survivors, a cancer diagnosis was established at 4 months of age at minimum, and at 8 years and 6 months at maximum. Twenty-eight patients were diagnosed with the following solid tumors: nephroblastoma, neuroblastoma, anaplastic ependymoma, medulloblastoma, sarcoma granulocyticum, teratoma malignum, embryonal rhabdomyosarcoma, primitive neuroectodermal tumor (PNET)/Ewing sarcoma (ES), yolk sac tumor, clear cell sarcoma, astrocytoma pilocyticum, hepatoblastoma, and infantile fibrosarcoma. The remaining 9 participants suffered from different types of hematological neoplasms, predominantly leukemia. All of the survivors received

multi-agent chemotherapy, with the most frequently used drugs being VCR, DXR, CP, IF, VP-16, CBDCA, CDDP, ActD, daunorubicin (DNR), dacarbazine (DTIC), cytarabine (ARA-C), methotrexate (MTX), and mercaptopurine (6-MP). Surgery was required in 27 patients and additional radiotherapy was performed in 11 participants (cranial radiotherapy in 4 cases). After a detailed analysis, the effects of radiotherapy, including cranial radiation, were considered to be negligible for the purpose of this study. Dental examinations supplemented with a thorough analysis of panoramic radiographs revealed a total of 236 teeth with 243 different developmental abnormalities. Special attention was paid to agenesis, microdontia, tooth size reduction, taurodontia, and enamel and root abnormalities. Enamel abnormalities, such as opacities, hypoplasia and marked perikymata, were found in the majority of teeth. Agenesis was the rarest abnormality in the study cohort. Table 1 shows the results of dental examinations, including the prevalence of particular developmental changes, in total and for each analyzed drug. The patients in whom all teeth showed a reduced size (microdontia and reduction) were additionally analyzed as a subgroup (M+R) due to the same mechanism of origin and the expected longer treatment duration. Some survivors presented with other dental abnormalities, such as root changes or supernumerary teeth. However, the small number of affected patients made statistical analysis impossible and these abnormalities were not further considered in this study.

A detailed analysis of the patients' medical records was also performed. The following treatment protocols were implemented in the study group: CWS 2006 Non-RMS-like HRG; CWS 2006 RMS-like; CWS 2002 (HR); SIOP 2001; 2002 PPGL; PPGGL SIOP December 2001; Euro-Ewing 99 PPGGL; Protocol I, III, IV PPGGL; Protocol II, III, IV PLGM recommended by PPGGL; TGM 95 PPGGL (HR); SIOPEL 3 (SR); ALL IC-BFM 2002 (SR, IR); ALL-REZ BFM 2002; and BFM Interim 2004 HRG. Among these protocols, VCR administration was the most common, followed by alkylating agent-containing anticancer therapy (CP, IF), anthracycline antibiotic-containing regimens (DXR, DNR), platinum-based chemotherapy (CBDCA, CDDP), and ActD. The characteristics of the study population and important treatment details are summarized in Table 1.

The next step was to analyze the mean treatment duration and the average cumulative dose for the most frequently used anticancer drugs, which were calculated separately for different abnormalities in relation to patients with or without the analyzed abnormality, and in relation to patients without dental abnormalities. The relationship between the treatment duration and the occurrence of particular disturbances for each drug was also assessed. The same procedure was applied for the cumulative drug doses administered during the first 10 weeks of treatment and for the entire duration of the therapy using the analyzed drug (Table 2).

Statistical analysis

The patient characteristics were analyzed using the non-parametric Mann–Whitney *U* test. Continuous variables were reported as the mean (*M*), standard deviation (*SD*), minimum (min), and maximum (max) values. The statistical analysis of the treatment period data involved the correlation between drug administration in the affected and/or non-affected survivors and the prevalence of long-term dental effects, and the impact of age at the onset of therapy and the treatment duration on the occurrence of abnormalities. Radiographically and clinically recorded dental outcomes, such as agenesis, microdontia, reduction in tooth size, the M+R status, enamel changes, and taurodontia, were assessed in this analysis. The values were reported in weeks and milligrams. To assess differences and the strength of the relationships between the chemotherapy-related variables analyzed in Table 2, the Mann–Whitney *U* test and Spearman's rho were used, respectively (Table 2 and Table 3). A p-value ≤0.05 was considered to be statistically significant. All statistical analyses were performed using the Statistica® software, v. 13.3 (StatSoft Polska, Kraków, Poland).

Results

The results are shown in Tables 1–3. All patients (100%) treated with CDDP were diagnosed with dental developmental changes. However, this rate was not significantly higher than that for other anticancer drugs. The prevalence of the affected patients treated with various medications analyzed separately was comparable to the prevalence noted in all treated participants (Table 1). The age at diagnosis for each drug or group of medications in the total population and in the affected cancer survivors ranged between 4 and 102 months, whereas the non-affected cancer survivors started their treatment at a minimum of 25 months and a maximum of 69 months (Table 1).

With regard to the administration of VCR, antibiotics and VP-16, treatment with alkylating and platinum-based agents was paradoxically longer in the non-affected patients than the affected ones (Table 2). In order to establish the treatment duration-dependency of different long-term dental effects, the mean duration of anticancer therapy was determined for 6 groups of survivors diagnosed with agenesis, microdontia, tooth size reduction, microdontia and/or tooth size reduction, enamel defects, and taurodontia. Due to the combined drug administration in some anticancer protocols, a few drug analogs were excluded from a detailed analysis. No significant differences in the treatment duration were noted between abnormalities within the affected and non-affected groups for each analyzed medication, except for patients with a reduction in tooth size and enamel changes in the CP affected group ($p = 0.05$) (Table 2). Furthermore, there were no significant differences in the treatment duration

Table 1. Study group characteristics

Drug/ group of drugs (prevalence of patients treated [%])	Number of patients			Mean age at the treatment onset [months] min–max			Mean duration of treatment with drug analyzed [weeks] *M* ±*SD* min–max			Mean duration of treatment with all medications [weeks] *M* ±*SD* min–max			Number of teeth affected						
	total *n* (%)	affected *n* (%) n_A/1	non-affected *n* (%)	total	affected	non-affected	total	affected	non-affected	total	affected	non-affected	total *n* (n_A)	agenesis n_A (%) *p*-value	microdontia n_A (%) *p*-value	reduction in tooth size n_A (%) *p*-value	enamel changes n_A (%) *p*-value	taurodontia n_A (%) *p*-value	root changes n_A (%) *p*-value
Total (100.00)	37 (100.00)	28 (75.68) 8.7/1	9 (24.32)	38.08 4–102	35.07 4–102	47.44 25–69	–	–	–	60.49 ±32.65 5–122	60.75 ±31.23 11–122	59.67 ±38.81 5–118	236 (243)	20 (8.23)	30 (12.35)	59 (24.28)	80 (32.92)	27 (11.11)	27 (11.11)
VCR (81.08)	30 (100.00)	22 (73.33) 9.3/1	8 (26.67)	36.40 4–91	32.45 4–91	47.25 25–69	38.10 ±20.06 5–83	38.50 ±23.09 6–83	37.00 ±20.38 5–63	64.77 ±32.80 5–122	66.27 ±30.10 11–122	60.63 ±41.38 5–118	198 (205)	18 (8.78)	19 (9.27)	53 (25.85)	67 (32.68)	21 (10.24)	27 (13.17)
DXR (35.14)	13 (100.00)	11 (84.62) 8.4/1	2 (15.38)	29.85 4–72	27.73 4–72	41.50 37–46	24.85 ±12.82 1–47	27.55 ±13.19 7–47	10.00 ±12.73 1–19	50.69 ±22.09 5–88	54.36 ±18.95 30–88	30.50 ±36.06 5–56	88 (92)	16 (17.39) 0.0300*	18 (19.57)	13 (14.13)	30 (32.61)	14 (15.22)	1 (1.09) 0.0060*
DXR/DNR (54.05)	20 (100.00)	16 (80.00) 8.0/1	4 (20.00)	34.70 4–91	32.25 4–91	44.50 37–48	26.20 ±11.74 1–47	27.31 ±10.85 7–47	21.75 ±15.86 1–38	69.65 ±34.17 5–122	69.38 ±30.88 30–122	70.75 ±51.25 5–118	124 (128)	20 (15.63) 0.0400*	19 (14.84)	32 (25.00)	30 (23.44)	16 (12.50)	11 (8.59)
CP (35.14)	13 (100.00)	11 (84.62) 9.8/1	2 (15.38)	34.85 11–91	32.55 11–91	47.50 47–48	28.46 ±6.02 20–40	28.91 ±6.43 20–40	26.00 ±1.00 25–27	81.08 ±32.46 39–122	75.64 ±32.29 39–122	111.00 ±9.90 104–118	103 (108)	8 (7.41)	21 (19.44)	28 (25.93)	32 (29.63)	9 (8.33)	10 (9.26)
IF (21.62)	8 (100.00)	7 (87.50) 8.9/1	1 (12.50)	38.00 4–88	38.14 4–88	37.00 37–37	23.88 ±14.01 4–49	20.29 ±10.44 4–32	49.00 ±0.00 49–49	59.38 ±25.84 17–88	59.86 ±27.87 17–88	56.00 ±0.00 56–56	60 (62)	9 (14.52)	6 (9.68)	12 (19.35)	11 (17.74) 0.0200*	7 (11.29)	17 (27.42) 0.0020*
CP/IF (56.76)	21 (100.00)	18 (85.71) 9.4/1	3 (14.29)	36.05 4–91	34.72 4–91	44.00 37–48	26.71 ±9.87 4–49	25.56 ±9.16 4–40	33.67 ±13.32 25–49	72.81 ±31.34 17–122	69.50 ±30.83 17–122	92.67 ±32.52 56–118	162 (170)	17 (10.00)	27 (15.88)	40 (23.53)	43 (25.29)	16 (9.41)	27 (15.88)
VP-16 (37.84)	14 (100.00)	12 (85.71) 8.6/1	2 (14.29)	30.31 4–102	34.42 4–102	41.50 37–46	21.71 ±12.00 1–39	23.33 ±11.88 1–39	12.00 ±9.90 5–19	52.36 ±24.54 5–82	56.00 ±22.18 17–82	30.50 ±36.06 5–56	100 (103)	8 (7.77)	23 (22.33) 0.0200*	17 (16.50)	29 (28.16)	10 (9.71)	16 (15.53)
CBDCA (35.14)	13 (100.00)	10 (76.92) 5.3/1	3 (23.08)	32.62 4–69	24.90 4–55	58.33 46–69	37.38 ±20.82 1–87	35.50 ±23.47 1–87	43.67 ±6.81 36–49	55.08 ±18.53 30–92	52.50 ±17.08 30–83	63.67 ±24.54 49–92	53 (53)	8 (15.09)	16 (30.19) 0.0020*	16 (30.19)	3 (5.66) 0.0001*	10 (18.87)	0 (0.00) 0.0200*
CDDP (18.92)	7 (100.00)	7 (100.00) 10.9/1	0 (0.00)	45 11–95	45 11–95	–	34.29 ±19.32 14–69	34.29 ±19.32 14–69	–	49.00 ±30.51 14–80	49.00 ±30.51 14–80	–	71 (76)	0 (0.00) 0.0200*	12 (15.79)	11 (14.47)	29 (38.16)	8 (10.53)	16 (21.05) 0.0200*
CBDCA/ CDDP (54.05)	20 (100.00)	17 (85.00) 7.6/1	3 (15.00)	36.95 4–95	33.18 4–95	58.33 46–69	36.30 ±19.85 1–87	35.00 ±21.22 1–87	43.67 ±6.81 36–49	52.95 ±22.79 14–92	51.06 ±22.72 14–83	63.67 ±24.54 49–92	124 (129)	8 (6.20)	28 (21.71) 0.0100*	27 (20.93)	32 (24.81)	18 (13.95)	16 (12.40)
ActD (32.43)	12 (100.00)	10 (83.33) 10.5/1	2 (16.67)	27.33 9–55	26.6 9–55	31 25–37	18.25 ±13.73 3–43	18.50 ±14.68 3–43	17.00 ±11.31 9–25	43.08 ±21.17 11–88	45.00 ±20.27 11–88	33.50 ±31.82 11–56	101 (105)	14 (13.33)	11 (10.48)	13 (12.38) 0.010*	54 (51.43) 0.001*	12 (11.43)	1 (0.95) 0.002*

n – number of patients/number of teeth affected; n_A – number of abnormalities (e.g., 2 abnormalities in 1 tooth); n_A/1 – number of abnormalities per 1 affected patient; min – minimum; max – maximum; *M* – mean; SD – standard deviation; VCR – vincristine; DXR – doxorubicin; DNR – daunorubicin; CP – cyclophosphamide; IF – ifosfamide; VP-16 – etoposide; CBDCA – carboplatin; CDDP – cisplatin; ActD – actinomycin-D;
* statistically significant difference between the drug group and the total group ($p \leq 0.05$; Mann–Whitney *U* test).

between the affected and non-affected patients within most groups of dental abnormalities for each drug, except for microdontia in the DXR group ($p = 0.04$) and a reduction in tooth size in the CP group ($p = 0.03$) (Table 2). However, CP therapy in the participants with a reduced crown size was paradoxically longer in the non-affected group, with a similar anomaly appearing in many groups of abnormalities regardless of the drug administered (Table 2). For the mean treatment duration, no significant correlations were observed in the VCR group. Strong significant positive correlations in the affected group were observed between microdontia and M+R in CP recipients ($p = 0.01$), M+R and the total group receiving VP-16 ($p = 0.01$), and taurodontia and the total group receiving CDDP ($p = 0.01$). Among the non-affected patients with a particular abnormality, the following strong significant positive correlations were noted: agenesis vs. reduction in tooth size in DXR recipients ($p = 0.0003$); agenesis vs. taurodontia in the CP group ($p = 0.0005$); agenesis vs. enamel changes in the IF and CBDCA groups ($p = 0.004$ and $p = 0.0003$, respectively); and M+R vs. agenesis ($p = 0.03$) and enamel changes ($p = 0.03$) in patients receiving CBDCA. A strong significant positive correlation was also established between the affected and non-affected patients with a particular abnormality within the DXR group in terms of reduction in tooth size ($p = 0.05$). Strong significant negative correlations were established only within the ActD group between the affected and non-affected patients in terms of reduction in tooth size and taurodontia ($p = 0.05$ and $p = 0.05$, respectively). Some groups of patients with disturbed odontogenesis were too small to analyze the relationships (Table 3).

To test the hypothesis that the observed shorter treatment intervals in the first 10 weeks would result in a higher cumulative treatment dose than in the remaining longer period of therapy, a separate calculation was performed for the initial 10 weeks and for the total duration of therapy. There were no statistically significant differences between patients with different disturbances in most specific drug groups when analyzing the treatment duration and the drug doses, except for VCR patients with agenesis and microdontia in the first 10 weeks of therapy. Moreover, a pattern was frequently observed such that the mean treatment dose in the affected group was lower than in the non-affected group, although no statistically significant differences were noted, except for the following groups: VCR (the first 10 weeks of therapy) presenting with microdontia ($p = 0.01$); IF exhibiting M+R (both the initial 10 weeks and the total duration of therapy) ($p = 0.02$ in both cases); and the CBDCA total group (both the initial 10 weeks and the total duration of therapy) ($p = 0.03$ and $p = 0.05$, respectively). In all of the above examples, the mean cumulative treatment dose was significantly higher in the groups non-affected with the analyzed abnormalities (Table 2). Due to the lack of differences in the treatment duration and dose, only the correlations between the survivors with different abnormalities, and between the survivors affected and non-affected with a particular abnormality could be established. Strong significant correlations are listed in Table 3.

Discussion

The toxic effects of chemotherapy on odontogenesis have been well documented in animal models.[9,23,24] Cell susceptibility to toxic factors varies depending on the developmental stage during the course of treatment, and is related to different side effects, from the death of immature precursors, through transient development cessation, to the disturbed metabolic function of fully formed elements. Phenotypically, the toxic effects of chemotherapy can be observed as the lack of or reduced germs, abnormal tissue structure, or shorter dental roots.[19,25–28]

Clinical risk factors, such as age at diagnosis, the stage of dental development at the onset of treatment and the type of therapy, are still areas of active research.[10,14,20,25] A young age at diagnosis is hypothesized to be the most important risk factor. It is widely documented that the earlier anticancer therapy is started, the higher probability of dental abnormalities. The most affected patients have been reported to be treated before 3–5 years of age and they tended to show the most severe abnormalities, such as agenesis and microdontia.[3,7,11,14–19] In our study, only one survivor started treatment before 3 years of age, and presented with no abnormalities and a relatively short therapy duration. This patient received 8 courses of VCR and 3 cycles of ActD during 11 weeks of chemotherapy. However, as shown in an earlier study, agenesis and a reduction in crown size were also diagnosed in patients not treated at a very young age.[25] If therapy overlaps with the early odontogenesis of third molars, which develop at a later age, agenesis or microdontia may also occur.[25] Some investigators reported no significant relationship between tooth impairment and age at the onset of therapy, although some noticed an association between early exposure to chemotherapy and agenesis or microdontia.[7,11,17] Proc et al. reported that microdontia was observed in patients who started therapy before 42 months of age; patients treated between 43 and 61 months of age had no abnormalities, and patients aged >61 months presented with disturbed third molars.[11] In our study, the use of drug-dependent group division randomly created various age groups of patients. Agenesis was most commonly diagnosed (more often than the average prevalence of 8.23%) in younger affected patients, aged between 24.90 and 38.14 months at the onset of anticancer treatment. Combined M+R was more common (vs. the average prevalence of 36.63%) when therapy was administered to patients aged between 24.90 and 34.72 months. A higher than average (11.11%) prevalence of root disturbances was observed in the older group of patients who started

Table 2. Characteristics of the groups affected and non-affected with particular abnormalities in terms of treatment duration and dose administered for each analyzed drug

Drug administered (group analyzed)	Mean treatment duration [weeks] *M ±SD*							Mean cumulative drug dose 0–10 weeks [mg] *M ±SD*							Mean cumulative drug dose 0–90 weeks [mg] *M ±SD*						
	total	agenesis	microdontia	reduction in tooth size	M+R	enamel changes	taurodontia	total	agenesis	microdontia	reduction in tooth size	M+R	enamel changes	taurodontia	total	agenesis	microdontia	reduction in tooth size	M+R	enamel changes	taurodontia
VCR (affected)	38.50 ± 23.09	25.20 ± 9.96	39.50 ± 23.63	39.93 ± 21.55	41.18 ± 22.95	28.20 ± 21.34	46.00 ± 22.95	5.28 ± 2.41	**5.90 ± 2.06[d]**	**3.71 ± 2.09[de]**	5.09 ± 2.50	4.85 ± 2.48	4.19 ± 2.68	4.72 ± 2.72	12.47 ± 8.24	9.56 ± 3.64	10.87 ± 10.29	10.92 ± 5.19	11.81 ± 7.76	7.47 ± 1.55	12.32 ± 9.01
VCR (non-affected)	37.00 ± 20.38	40.68 ± 23.02	37.59 ± 22.02	36.50 ± 23.07	34.08 ± 21.05	40.08 ± 22.08	34.71 ± 21.33	6.55 ± 3.65	5.56 ± 2.94	**6.40 ± 2.78[e]**	6.07 ± 3.02	6.61 ± 2.94	5.70 ± 2.86	6.00 ± 2.79	12.81 ± 7.87	13.16 ± 8.54	13.17 ± 7.19	13.99 ± 9.79	13.54 ± 8.53	13.58 ± 8.40	12.66 ± 7.77
VCR (total)	38.10 ±20.06							5.60 ±2.76							12.56 ±7.89						
DXR (affected)	27.55 ± 13.19	27.60 ± 11.26	**34.80 ± 8.56[a]**	20.75 ± 6.08	29.29 ± 11.73	35.67 ± 17.10	26.40 ± 12.03	71.32 ± 48.12	68.94 ± 50.86	45.97 ± 34.16	79.92 ± 52.12	65.41 ± 46.69	104.07 ± 74.42	44.51 ± 38.15	153.28 ± 68.04	155.48 ± 68.51	159.64 ± 54.34	153.08 ± 66.44	157.46 ± 50.16	194.8 ± 66.34	113.52 ± 71.30
DXR (non-affected)	10.00 ± 12.73	23.13 ± 16.28	**18.63 ± 13.76[a]**	26.67 ± 16.64	19.67 ± 16.12	21.60 ± 12.39	24.25 ± 15.73	69.20 ± 54.87	72.28 ± 47.50	86.64 ± 48.44	67.03 ± 46.86	77.52 ± 50.25	61.08 ± 34.70	87.55 ± 45.76	123.20 ± 131.24	144.39 ± 80.98	141.79 ± 86.40	146.69 ± 80.39	138.38 ± 98.77	134.81 ± 72.99	170.61 ± 70.40
DXR (total)	24.85 ±12.82							71.00 ±46.71							148.65 ±73.62						
CP (affected)	28.91 ± 6.43	30.00 ± 7.94	31.17 ± 5.19	**25.29 ± 5.22[bc]**	27.80 ± 5.96	**35.00 ± 4.36[c]**	29.25 ± 6.18	885.85 ± 500.29	940.00 ± 225.17	650.40 ± 361.21	850.00 ± 339.12	775.24 ± 346.89	885.80 ± 995.61	520.60 ± 343.59	2719.21 ± 1286.68	3300.00 ± 1530.88	2741.47 ± 1251.62	2207.14 ± 626.78	2484.88 ± 1080.91	3070.43 ± 1891.51	2012.20 ± 735.66
CP (non-affected)	26.00 ± 1.00	28.00 ± 6.11	26.14 ± 6.52	**32.17 ± 5.56[b]**	30.67 ± 8.14	26.50 ± 5.44	28.11 ± 6.64	375.00 ± 530.33	770.74 ± 583.43	946.43 ± 618.54	762.90 ± 709.44	925.00 ± 1023.78	787.00 ± 369.08	938.33 ± 546.99	2200.00 ± 141.42	2441.13 ± 1087.41	2551.79 ± 1230.35	3143.55 ± 1538.93	3154.17 ± 1655.69	2510.00 ± 1008.37	2918.06 ± 1281.39
CP (total)	28.46 ±6.02							809.8 ±518.89							2639.33 ± 1191.34						
IF (affected)	20.29 ± 10.44	26.00 ± 1.41	17.33 ± 14.05	28.33 ± 3.51	21.00 ± 11.18	25.00 ± 0.00	19.33 ± 11.37	10.89 ± 7.96	8.40 ± 1.70	7.75 ± 1.10	7.85 ± 1.00	**7.56 ± 0.82[f]**	7.20 ± 0.00	14.92 ± 12.06	22.83 ± 12.20	34.20 ± 12.73	14.91 ± 10.59	23.35 ± 4.85	**17.56 ± 8.71[h]**	25.20 ± 0.00	22.08 ± 10.12
IF (non-affected)	49.00 ± 0.00	23.17 ± 16.50	27.80 ± 13.92	21.20 ± 17.71	28.67 ± 19.55	23.71 ± 15.13	26.60 ± 15.95	16.20 ± 0.00	12.60 ± 8.67	13.83 ± 9.13	13.77 ± 9.19	**18.20 ± 9.75[f]**	12.17 ± 8.00	9.53 ± 3.86	45.00 ± 0.00	22.73 ± 13.89	32.01 ± 11.74	26.95 ± 17.69	**39.00 ± 8.88[h]**	25.65 ± 14.85	27.71 ± 16.27
IF (total)	23.88 ±14.01							11.55 ±7.61							25.60 ±13.75						

Drug administered (group analyzed)	Mean treatment duration [weeks] *M* ±*SD*							Mean cumulative drug dose 0–10 weeks [mg] *M* ±*SD*							Mean cumulative drug dose 0–90 weeks [mg] *M* ±*SD*						
	total	agenesis	microdontia	reduction in tooth size	M+R	enamel changes	taurodontia	total	agenesis	microdontia	reduction in tooth size	M+R	enamel changes	taurodontia	total	agenesis	microdontia	reduction in tooth size	M+R	enamel changes	taurodontia
VP-16 (affected)	23.33 ± 11.88	22.00 ± 18.19	26.00 ± 9.74	24.83 ± 9.50	24.10 ± 9.62	32.00 ± 11.27	28.25 ± 9.74	261.25 ± 285.57	210.00 ± 187.35	330.00 ± 315.56	180.00 ± 173.12	277.50 ± 301.19	240.00 ± 226.50	232.50 ± 179.37	1121.88 ± 731.84	1380.00 ± 923.69	1193.44 ± 505.69	785.00 ± 338.97	1040.25 ± 553.17	1660.00 ± 905.15	864.38 ± 268.14
VP-16 (non-affected)	12.00 ± 9.90	21.64 ± 10.99	16.00 ± 13.15	19.38 ± 13.72	15.75 ± 16.72	18.91 ± 11.01	19.10 ± 12.23	817.50 ± 10.61	376.36 ± 359.47	355.00 ± 381.68	461.25 ± 379.20	498.75 ± 396.37	368.18 ± 358.65	384.00 ± 374.98	1222.50 ± 562.15	1069.77 ± 655.11	1060.00 ± 935.29	1399.69 ± 788.76	1376.25 ± 1024.43	993.41 ± 594.89	1245.00 ± 788.48
VP-16 (total)	21.71 ±12.00							340.71 ±331.38							1136.25 ±691.98						
CBDCA (affected)	35.50 ± 23.47	21.33 ± 17.62	34.00 ± 9.90	42.33 ± 26.27	39.63 ± 22.77	37.00 ± 0.00	48.50 ± 27.97	**444.60 ± 528.48[g]**	240.00 ± 207.85	333.20 ± 433.63	621.00 ± 614.62	510.75 ± 566.36	–	684.00 ± 671.10	**2532.10 ± 1783.00[i]**	1800.00 ± 1298.00	2137.20 ± 1202.58	2720.17 ± 2111.01	2670.13 ± 1796.87	3600.00 ± 0.00	2827.75 ± 2292.78
CBDCA (non-affected)	43.67 ± 6.81	42.20 ± 19.94	39.50 ± 25.96	33.14 ± 15.70	33.80 ± 19.18	37.42 ± 21.75	32.44 ± 16.36	**1358.33 ± 562.86[g]**	780.10 ± 690.08	856.88 ± 701.99	685.00 ± 723.82	887.00 ± 768.86	710.08 ± 645.27	723.13 ± 678.74	**4816.67 ± 1172.36[i]**	3437.10 ± 1939.38	3635.63 ± 2094.61	3350.00 ± 1818.51	3682.00 ± 2100.81	3014.25 ± 1979.18	3467.50 ± 1716.10
CBDCA (total)	37.38 ±20.82							655.46 ±648.43							3059.31 ±1901.87						
CDDP (affected)	34.29 ± 19.32	–	41.75 ± 21.72	26.50 ± 17.68	36.20 ± 22.53	41.00 ± 2.83	31.40 ± 12.05	138.79 ± 155.05	–	93.13 ± 58.32	94.00 ± 56.57	101.30 ± 53.71	45.25 ± 12.34	135.10 ± 189.48	432.13 ± 344.22	–	333.48 ± 248.33	192.00 ± 8.49	303.98 ± 224.95	228.95 ± 43.77	427.38 ± 379.93
CDDP (non-affected)	–	34.29 ± 19.32	24.33 ± 12.34	37.40 ± 20.96	29.50 ± 12.02	31.60 ± 22.94	41.50 ± 38.89	–	138.79 ± 155.05	199.67 ± 239.35	156.70 ± 184.00	234.00 ± 330.93	176.20 ± 172.91	148.00 ± 19.80	–	432.13 ± 344.22	563.67 ± 466.40	528.18 ± 370.60	752.50 ± 470.23	513.40 ± 385.16	444.00 ± 364.87
CDDP (total)	34.29 ±19.32							138.79 ±155.05							432.13 ±344.21						
ActD (affected)	18.50 ± 14.68	21.25 ± 12.34	14.25 ± 19.21	26.00 ± 15.85	18.67 ± 16.74	10.00 ± 8.37	21.75 ± 20.22	1538.40 ± 585.94	1361.25 ± 476.45	1425.00 ± 695.52	1391.25 ± 659.02	1415.00 ± 623.30	1543.50 ± 754.73	1248.75 ± 570.97	3506.40 ± 2207.54	4121.25 ± 1642.50	2010.00 ± 820.76	4428.75 ± 2706.25	3455.00 ± 2603.28	2466.00 ± 1474.66	2493.75 ± 1276.34
ActD (non-affected)	17.00 ± 11.31	16.75 ± 14.94	20.25 ± 11.15	14.38 ± 11.70	17.83 ± 11.57	22.38 ± 14.41	16.50 ± 10.50	799.00 ± 1129.96	1442.13 ± 808.83	1410.25 ± 739.31	1427.13 ± 753.24	1415.33 ± 816.75	1351.00 ± 704.09	1498.38 ± 768.46	2374.00 ± 1097.43	2915.88 ± 2243.49	3971.50 ± 2233.50	2762.13 ± 1595.63	3180.33 ± 1617.96	3743.50 ± 2277.99	3729.63 ± 2337.29
ActD (total)	18.25 ±13.73							1415.17 ±692.68							3317.67 ±2071.46						

M+R – microdontia + reduction in tooth size subgroup. The same superscript letters indicate a statistically significant difference ($p \leq 0.05$; Mann–Whitney *U* test).

Table 3. Strong correlations according to Spearman's rho with regard to the study results depicted in Table 2

Drug administered	Value analyzed	Group tested	Therapy duration [weeks]	Group of patients with the same adverse effect						
				agenesis (A)	microdontia (M)	reduction in size (R)	M+R	enamel changes (E)	taurodontia (T)	total
VCR	treatment duration	affected	X	T: $r_s = -0.80$ R: $r_s = 1$	T: $r_s = -0.69$*	–	T: $r_s = 0.65$*	–	total: $r_s = -0.65$*	$r_s = 1$
	dose	affected	0–10	R: $r_s = -0.90$*	–	–	T: $r_s = 0.82$*	–	–	$r_s = 1$
			0–90	T: $r_s = 0.80$	–	–	T: $r_s = 0.93$*	–	–	$r_s = 1$
		non-affected	0–10	total: $r_s = 0.71$*	–	–	E: $r_s = -0.57$*	–	total: $r_s = -0.78$*	–
			0–90	–	$r_s = -0.76$*	–	–	–	–	–
DXR	treatment duration	affected	X	E: $r_s = 1$	–	M+R, total: $r_s = 0.83$	–	T: $r_s = -0.86$	–	–
		non-affected	X	R: $r_s = 0.94$* E: $r_s = 0.71$* T: $r_s = 0.70$*	–	–	–	T: $r_s = 0.75$*	–	–
		affected/non-affected	X	–	–	$r_s = 0.94$*	–	–	–	–
	dose	affected	0–10	R: $r_s = -0.80$	R: $r_s = 0.80$ E: $r_s = 0.86$	–	E: $r_s = -1$	–	–	$r_s = 1$
			0–90	–	–	total: $r_s = 0.80$	T: $r_s = 0.80$	–	–	$r_s = 1$
		non-affected	0–10	M+R: $r_s = -0.82$*	–	–	–	–	–	–
			0–90	T: $r_s = 0.68$*	–	–	–	–	–	–
		affected/non-affected	0–10	–	–	–	$r_s = 0.80$	–	$r_s = 0.80$	–
CP	treatment duration	affected	X	M, M+R: $r_s = -0.86$ E: $r_s = 1$	M+R: $r_s = 0.89$* E: $r_s = -0.86$	E: $r_s = 0.86$	E: $r_s = -0.86$ total: $r_s = 0.62$*	–	–	$r_s = 1$
		non-affected	X	M+R: $r_s = -1$ T: $r_s = 0.91$*	M+R: $r_s = -1$	–	E: $r_s = 1$	–	–	–
		affected/non-affected	X	$r_s = 0.86$	–	–	–	–	–	–
	dose	affected	0–10	E: $r_s = 0.86$ total: $r_s = -1$	E: $r_s = -0.86$	E: $r_s = -0.86$	E: $r_s = -0.86$	T, total: $r_s = -0.86$	–	$r_s = 1$
			0–90	M, M+R: $r_s = 1$ E: $r_s = -1$	M+R: $r_s = 1$ E: $r_s = -1$ T: $r_s = -0.80$	T: $r_s = 1$	E: $r_s = -1$ T: $r_s = -0.80$	–	–	$r_s = 1$
		non-affected	0–10	M+R: $r_s = 0.86$	–	M+R: $r_s = -0.86$	T: $r_s = 0.86$	–	–	–
			0–90	–	M+R: $r_s = -0.86$ E: $r_s = -0.78$*	M+R: $r_s = -1$	T: $r_s = -1$	–	–	–
		affected/non-affected	0–10	–	–	–	$r_s = 0.86$	–	$r_s = -0.86$	–
			0–90	–	–	–	$r_s = 1$	–	$r_s = 0.80$	–

Drug administered	Value analyzed	Group tested	Therapy duration [weeks]	Group of patients with the same adverse effect						
				agenesis (A)	microdontia (M)	reduction in size (R)	M+R	enamel changes (E)	taurodontia (T)	total
VP-16	treatment duration	affected	X	M, E, T: $r_s = 1$ R, M+R, total: $r_s = -1$	M+R, total: $r_s = -0.77$* E, T: $r_s = 1$	M+R, total: $r_s = 1$ E: $r_s = -1$ T: $r_s = -0.80$	E: $r_s = -1$ T: $r_s = -0.80$ total: $r_s = 0.81$*	T: $r_s = 1$ total: $r_s = -1$	total: $r_s = -0.80$	$r_s = 1$
		non-affected	X	–	M+R: $r_s = -0.80$	–	–	–	–	–
		affected/non-affected	X	$r_s = -1$	–	–	–	$r_s = -1$	$r_s = -0.80$	–
	dose	affected	0–10	R, M+R, total: $r_s = 1$	T: $r_s = 1$	M+R, total: $r_s = 1$	total: $r_s = 0.66$*	–	–	$r_s = 1$
			0–90	M, T: $r_s = 1$	T: $r_s = 1$	M+R, total: $r_s = 0.82$* E: $r_s = -1$	E: $r_s = -1$ total: $r_s = 0.64$*	total: $r_s = -1$	–	$r_s = 0.97$*
		non-affected	0–10	–	–	–	E: $r_s = 0.94$* T: $r_s = -0.94$*	–	–	–
			0–90	–	E: $r_s = 0.82$*	–	–	–	–	–
		affected/non-affected	0–10	$r_s = 1$	–	–	–	$r_s = -0.86$	–	–
			0–90	–	–	–	–	$r_s = -1$	–	–
IF	treatment duration	affected	X	–	T: $r_s = 1$	M+R, total: $r_s = 1$	total: $r_s = 1$	–	–	$r_s = 1$
		non-affected	X	E: $r_s = 0.94$*	T: $r_s = 1$	–	–	–	–	–
	dose	affected	0–10	–	–	M+R, total: $r_s = 1$ T: $r_s = -1$	T: $r_s = -1$	–	total: $r_s = -1$	$r_s = 1$
			0–90	–	–	–	T: $r_s = -1$	–	total: $r_s = -1$	$r_s = 1$
		non-affected	0–10	M+R: $r_s = -1$	M+R: $r_s = 1$	–	E: $r_s = -1$	–	–	–
			0–90	–	M+R: $r_s = -1$	–	–	–	–	–
		affected/non-affected	0–10	–	–	–	$r_s = -1$	–	–	–
			0–90	–	$r_s = -1$	$r_s = 1$	–	–	$r_s = 1$	–
CBDCA	treatment duration	affected	X	R, total: $r_s = -1$	T: $r_s = 0.80$	total: $r_s = 1$	–	–	–	$r_s = 1$
		non-affected	X	M+R, E: $r_s = 0.90$*	–	–	E: $r_s = 0.90$*	–	–	–
		affected/non-affected	X	$r_s = 1$	–	–	–	–	–	–
	dose	affected	0–10	M, T: $r_s = 0.86$	–	M+R, total: $r_s = 1$	total: $r_s = 0.89$*	–	–	$r_s = 1$
			0–90	R, M+R, total: $r_s = -1$	T: $r_s = 0.80$	M+R, total: $r_s = 1$	total: $r_s = 0.97$*	–	–	$r_s = 1$
		non-affected	0–10	total: $r_s = 0.86$	–	total: $r_s = 0.86$	–	total: $r_s = 0.86$	–	–
			0–90	E: $r_s = 0.75$*	–	–	–	–	–	–
		affected/non-affected	0–10	–	$r_s = 0.87$*	–	–	–	–	$r_s = 0.86$
			0–90	$r_s = -1$	–	–	–	–	–	–

Drug administered	Value analyzed	Group tested	Therapy duration [weeks]	Group of patients with the same adverse effect						
				agenesis (A)	microdontia (M)	reduction in size (R)	M+R	enamel changes (E)	taurodontia (T)	total
CDDP	treatment duration	affected	X	–	–	–	–	–	total: $r_s = 0.90$*	–
		non-affected	X	E: $r_s = -0.80$ T: $r_s = 1$	R: $r_s = -1$	–	–	total: $r_s = -0.80$	–	–
		affected/non-affected	X	–	–	–	–	–	–	$r_s = 1$
	dose	affected	0–10	–	M+R, total: $r_s = 0.80$	–	–	–	–	$r_s = 1$
			0–90	–	T: $r_s = 1$	–	total: $r_s = 1$	–	–	$r_s = 1$
		non-affected	0–10	M: $r_s = 1$	R: $r_s = -1$	E: $r_s = 0.90$*	–	–	–	$r_s = 0.86$
			0–90	–	–	E: $r_s = 1$	–	–	–	–
		affected/non-affected	0–10	–	$r_s = 1$	–	–	–	–	–
ActD	treatment duration	affected	X	M, E: $r_s = -1$ M+R: $r_s = -0.80$	M+R: $r_s = 0.80$ E: $r_s = 1$	–	E: $r_s = 0.80$	–	total: $r_s = 1$	$r_s = 1$
		non-affected	X	$r_s = -0.80$	–	$r_s = -0.94$*	–	–	$r_s = -0.94$*	–
	dose	affected	0–10	M, M+R: $r_s = -0.94$* T, total: $r_s = 0.80$	M+R: $r_s = 0.83$ E: $r_s = 0.88$ T, total: $r_s = -0.94$*	–	T: $r_s = -0.94$* total: $r_s = 0.94$*	–	total: $r_s = 1$	$r_s = 1$
			0–90	–	E: $r_s = 0.94$	M+R: $r_s = 1$ E: $r_s = 0.80$	E: $r_s = 0.80$	–	total: $r_s = 1$	$r_s = 1$
		non-affected	0–10	M: $r_s = 0.79$* E: $r_s = 0.70$*	–	–	–	–	–	–
			0–90	–	R: $r_s = 0.79$*	E: $r_s = 0.80$*	–	–	–	–
		affected/non-affected	0–10	$r_s = -0.94$*	$r_s = 0.94$*	–	–	–	–	–

r_s – strong correlations between the abnormalities analyzed (Spearman's rho); *statistical significance ($p \leq 0.05$).

therapy at 32.45–45.00 months. Enamel changes occurred in participants aged 26.60–45.00 months during chemotherapy, and taurodontia, which was predominantly diagnosed in permanent first molars, in patients aged 24.90–38.14 months (Table 1). Although these observations do not account for the impact of particular drugs, and thus their potential individual toxicity, it is interesting that the results described above correspond with the age which is expected to be a particularly susceptible period in tooth development. Similar observations about the average age at the onset of treatment were reported by Proc et al.; patients with 1 missing tooth had an average age of 23.40 ±13.50 months, patients with >1 missing tooth had an average age of 31.71 ±22.07 months, patients with 1 microdens had an average age of 17.00 ±0.00 months, patients with >1 microdens had an average age of 35.75 ±27.63 months, and patients with root abnormalities had an average age of 32.83 ±21.27 months.[11] In addition, Wilberg et al. noticed a significant association between the age at diagnosis ≤5 years and microdontia and enamel hypoplasia.[15]

In some previous investigations, the type of anticancer agent, the treatment dose or duration were considered possible risk factors for dental sequelae.[15,20,21] The current study revealed no differences in the prevalence of affected patients treated with all of the analyzed medications. The prevalence of affected patients evaluated separately for each drug was higher than the average for the total study group (75.68%), except for patients receiving VCR (73.33%) (Table 1). Some differences appeared when the prevalence of particular abnormalities was analyzed. For agenesis, no hypodontia was diagnosed in the group of patients treated with CDDP, whereas this abnormality accounted for 17.39% of all dental changes among participants receiving DXR. Such a difference in prevalence as compared to the total study group was statistically significant. However, this observation cannot be interpreted without taking into account the average age at treatment initiation. In the CDDP group, despite the fact that the therapy duration was relatively long (34.29 weeks), the affected participants were treated at a later age (an average of 45.00 months), when agenesis or tooth germ aplasia is usually not expected. In contrast, the younger average age of the affected survivors from the DXR group (27.73 months) might explain the significantly higher prevalence of hypodontia. Similarly, a younger average age at the onset of therapy (24.90 months) is the probable reason for the relatively higher number of teeth reduced in size (M+R) in the CBDCA group (60.38%). Moreover, CBDCA therapy had a longer duration (an average of 37.38 weeks), which is only slightly shorter as compared to that for VCR. However, the lowest prevalence of teeth with changes in crown size (22.86%) was found in the ActD group with younger participants, aged on average 26.60 months. This could be explained by the fact that treatment with ActD was the shortest in the total study cohort, with the average duration of 18.25 weeks. However, the fact that the largest number of teeth in the ActD group had enamel abnormalities, which can occur over a wide age range, is non-negligible and may randomly disrupt reliable observations. On the other hand, the highest prevalence of taurodontia and root disturbances found for DXR and CBDCA, and for IF and CDDP, respectively, corresponded with the period of the potential developmental susceptibility of the analyzed dental tissues (Table 1). A separate evaluation of the impact of individual drugs without a thorough analysis of other treatment factors cannot explain all important correlations and may lead to faulty conclusions. Some previous authors attempted to evaluate the drug-dependency of dental abnormalities. According to Wilberg et al., treatment with anthracyclines was significantly associated with the individual defect index (IDeI).[15] Krasuska-Sławińska et al. reported positive correlations between hypodontia, age at the onset of treatment and the use of VCR, DXR, CP, IF, and VP-16.[20] A positive correlation was also observed between microdontia and treatment with VCR, DXR, CP, IF, and VP-16, and between taurodontia and VCR administration. The same analysis using Spearman's rho revealed positive correlations between dental root resorption, age at diagnosis and therapy with VCR, CDDP, CP, IF, VP-16, and DXR. The prevalence of enamel defects was positively correlated with age at the onset of treatment and the use of VCR, platinum agents, CP, DXR, IF, and VP-16. However, no concomitant analysis for a higher number of variables was performed. Moreover, Krasuska-Sławińska et al. noted that VP-16, CDDP and IF had no proven toxic dental activity.[20] In our study, a relatively higher prevalence of affected patients was noted in groups receiving these drugs (85.71%, 100.00% and 87.50%, respectively). Furthermore, a comparable number of dental abnormalities per affected patient was diagnosed for almost all drug groups (ranging from 8.4 to 10.5), except for the CBDCA group (an average of 5.3 abnormalities per affected patient). Unexpectedly, group receiving CDDP, which is a CBDCA analog, presented with the highest number of abnormalities per patient (10.9). Further studies are thus needed due to the multi-drug nature of anticancer therapy. For example, the ActD group had the shortest treatment with this drug and the shortest duration of the entire therapy (18.25 weeks and 43.08 weeks, respectively), but the highest number of abnormalities per affected survivor, apart from CDDP. It is possible that a young age at the onset of chemotherapy was a more significant risk factor in this group (Table 1).

The duration of cytotoxic therapy seems to be another important risk factor. Although there were no significant differences in the prevalence of affected survivors between the drug groups, the mean treatment duration ranged widely from 18.25 ±13.73 to 38.10 ±20.06 weeks (Tables 1,2). Moreover, the mean duration of individual drug therapy in relation to particular late effects was not always compatible with the results for the mean therapy duration investigated for the total group. For instance,

a longer treatment duration was noted in patients without agenesis who received VCR. For microdontia, the same observation was made in patients treated with ActD; for reduction in tooth size, it was observed for DXR, CP and CDDP; for enamel defects, it was observed for VCR and ActD; and for taurodontia, it was observed for CDDP (Table 2). Based on these results, no relationships between the drug and the treatment duration and adverse dental effects could be confirmed. According to our previous research, treatment-induced dental abnormalities are likely in all patients who receive chemotherapy during tooth formation.[25] Other researchers agree with the observation that the number of dental abnormalities does not differ with regard to the therapy duration.[11,12,22] However, Krasuska-Sławińska et al. reported positive correlations between enamel changes, microdontia, agenesis, and DeI and the chemotherapy duration.[20]

It is necessary to consider whether the treatment duration is proportional to the cumulative dose received. In general, the longer the duration of therapy, the higher the cumulative dose administered. The size of a single dose depends on the patient's weight or body surface at the treatment time. Although weight and the body surface increase with age, the susceptibility of tooth germinative cells does not change. Notably, most cytostatic drugs have a short elimination half-life in human plasma. After a single intravenous dose of 0.7 mg/m^2 VCR in dogs, the distribution half-life and the plasma elimination half-life were 21.5 ±6.9 min and 47.6 ±14.2 min, respectively.[29] The plasma VCR level was observed to decrease significantly up to 10% within 30 min after administration and about 99% of the plasma drug content was cleared within 5 h after administration. Moreover, 120 min after intravenous injection, VCR was not detectable in plasma.[29] The dose-limiting neurotoxicity and short elimination half-life of plasma VCR can reduce its anticancer activity.[30] Regardless of the aforementioned data, VCR is toxic to the dividing immature cells, and even more so when its administration is repeated at regular intervals. Intensive therapy with weekly VCR may thus make the regeneration of dental immature cells impossible. Moreover, humans exhibit a lower rate of CP metabolism as compared to dogs, cats or mice.[31] In fully developed dental tissue-secreting cells, polarized microscopy has revealed regular incremental lines in dentin corresponding to intravenous chemotherapy injection.[28] This abnormality, although observable, does not change tooth morphology and cannot be radiologically diagnosed. Similarly, the transient cessation of dental root development may be difficult to differentiate. Another question is the relation of the weight- or body surface-dependent doses of cytotoxic drugs to the immature tooth germ. Developing dental tissues have similar susceptibility to toxins, but chemotherapy doses differ significantly. The obvious expectation is that the higher the dose administered, the more potential damage to developing cells. Some authors reported dose-dependent dental abnormalities. Krasuska-Sławińska et al. observed dose-dependency between all possible congenital disturbances and VCR administration, as well as between hypodontia, microdontia, root resorption, and enamel defects and therapy with DXR, CP and IF.[20] Kaste et al. revealed dose-dependency between the risk of occurrence of at least one tooth abnormality and alkylating agent therapy.[21] Furthermore, they reported a dose-dependent association between alkylating agents and ≥1 tooth anatomical abnormality, abnormal roots and crown shape, a reduction in size, and ≥1 missing tooth in cancer survivors diagnosed at <5 years of age. They also noted a striking association between the cumulative dose of CP and an increased risk of dental sequelae.[21] Another study revealed dose-dependency between CP and Holtta's defect index (HDI); specifically, the administration of a CP dose ≥7.5 mg/m^2 increased the HDI score by 13 as compared to patients not treated with CP.[32] However, other prospective studies failed to confirm these observations.[12,22] The authors of the present study also did not note any pattern regarding the cumulative chemotherapeutic dose and the type of dental sequelae. Additionally, it is worth pointing to the hypothesis that the initial 10 weeks of treatment concerns the intensive part of anticancer therapy due to shorter intervals between toxic drug administration in that period. However, the drug doses during the initial 10 weeks relative to the cumulative drug doses over the entire treatment period corresponds to the percentage ratio – 10 weeks to the average whole duration of treatment with the analyzed medication ×100%. During the initial 10 weeks (26.25% of the average total treatment duration), only VCR was used at a relatively higher (44.59%) mean dose as compared to the mean value for the entire treatment period. A striking dose-dependent significant positive association in the affected patients was found between M+R and taurodontia in the VCR group, reduction in tooth size vs. M+R and the total group in the VP-16 group, M+R and the total group in CBDCA recipients, and M+R and the total group in the ActD group. Strong dose-dependent significant negative correlations in the affected patients were revealed between agenesis and reduction in tooth size in VCR recipients, as well as agenesis vs. microdontia and M+R, microdontia vs. taurodontia and the total group, and M+R vs. taurodontia in the ActD group (Table 3). In the VCR group, the patients with agenesis received a significantly higher mean drug dose as compared to the patients affected with microdontia in the first 10 weeks. By contrast, no significant dose differences were observed between the affected patients treated with ActD, although the mean total cumulative dose administered to the patients with hypodontia was more than two-fold higher than the dose administered to the patients with microdontia (Table 2).

In the present study, the age range of patients treated with all the analyzed medications was wide (from 4–11 months to 55–102 months at the onset of therapy). However, there was no relationship between age

and the treatment duration. In oncology, the required treatment protocols are typically introduced according to recommendations for the type and severity of the disease rather than age at diagnosis. Kaste et al. showed no increased frequency of developmental issues beyond the age of 5 years at diagnosis, although patients were exposed to the highest levels of alkylating agents.[21] Similarly, Minicucci et al. noted a higher prevalence of dental sequelae in a group of younger patients, even though these patients received longer-lasting chemotherapy with less intensive protocols.[3] According to Proc et al., there was no relationship between age at the onset of anticancer treatment and the prevalence of missing teeth in their study, where agenesis was diagnosed in each tooth group mineralizing in different age periods.[11]

Limitations

A substantial limitation of this study is the small number of participants treated with different protocols. The multi-drug nature of anticancer treatment enables decreasing its toxicity to normal human cells. However, this makes the evaluation of the toxicity of a single therapeutic agent difficult.

Conclusions

In the present study, there were no significant differences between the groups of cancer survivors with particular dental abnormalities in terms of treatment duration and drug doses. This result indicates that there is some other reason for late adverse effects. The developmental stage of tooth formation during chemotherapy is likely to be the most important factor determining dental changes in children. It is believed that even a small dose of each anticancer drug can affect immature tissues. Given the different treatment protocols used and other confounding factors, such as a small sample size, and varying age and therapy duration, an analysis of a more homogenous group of survivors seems necessary.

Ethics approval and consent to participate

The study was approved by the Bioethics Committee at the Medical University of Silesia, Katowice, Poland, on February 25, 2013 and November 29, 2016 (KNW/0022/KB1/15/I/13 and KNW/0022/KB1/15/II/16, respectively). The caregivers of the participants provided informed written consent prior to the investigations.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Anna Jodłowska https://orcid.org/0000-0002-4911-1612
Lidia Postek-Stefańska https://orcid.org/0000-0002-6573-019X

References

1. Chao MW, Lai MJ, Liou JP, et al. The synergic effect of vincristine and vorinostat in leukemia in vitro and in vivo. *J Hematol Oncol*. 2015;8:82. doi:10.1186/s13045-015-0176-7
2. Patel AG, Kaufmann SH. How does doxorubicin work? *Elife*. 2012;1:e00387. doi:10.7554/eLife.00387
3. Minicucci EM, Lopes LF, Crocci AJ. Dental abnormalities in children after chemotherapy treatment for acute lymphoid leukemia. *Leuk Res*. 2003;27(1):45–50. doi:10.1016/s0145-2126(02)00080-2
4. Lu DF, Wang JS, Li C, et al. Actinomycin D inhibits cell proliferations and promotes apoptosis in osteosarcoma cells. *Int J Clin Exp Med*. 2015;8(2):1904–1911. PMID:25932119. PMCID:PMC4402766.
5. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: Molecular mechanisms of action. *Eur J Pharmacol*. 2014;740:364–378. doi:10.1016/j.ejphar.2014.07.025
6. Bang M, Kim DG, Gonzales EL, Kwon KJ, Shin CY. Etoposide induces mitochondrial dysfunction and cellular senescence in primary cultured rat astrocytes. *Biomol Ther (Seoul)*. 2019;27(6):530–539. doi:10.4062/biomolther.2019.151
7. Avşar A, Elli M, Darka O, Pinarli G. Long-term effects of chemotherapy on caries formation, dental development, and salivary factors in childhood cancer survivors. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2007;104(6):781–789. doi:10.1016/j.tripleo.2007.02.029
8. Mataki S. Comparison of the effect of colchicine and vinblastine on the inhibition of dentinogenesis of rat incisors. *Arch Oral Biol*. 1981;26(12):955–961. doi:10.1016/0003-9969(81)90103-5
9. Stene T, Koppang HS. Autoradiographic investigation of proliferative responses in rat incisor pulp after vincristine administration. *Scand J Dent Res*. 1980;88(2):96–103. doi:10.1111/j.1600-0722.1980.tb01200.x
10. Jodłowska A, Postek-Stefańska L. Tooth development in the light of chemotherapy-induced agenesis in cancer survivors. *J Stoma*. 2021;74(1):28–33. doi:10.5114/jos.2021.104695
11. Proc P, Szczepańska J, Skiba A, Zubowska M, Fendler W, Młynarski W. Dental anomalies as late adverse effect among young children treated for cancer. *Cancer Res Treat*. 2016;48(2):658–667. doi:10.4143/crt.2015.193
12. Marec-Berard P, Azzi D, Chaux-Bodard AG, Lagrange H, Gourmet R, Bergeron C. Long-term effects of chemotherapy on dental status in children treated for nephroblastoma. *Pediatr Hematol Oncol*. 2005;22(7):581–588. doi:10.1080/08880010500198848
13. Kaste SC, Hopkins KP, Bowman LC, Santana VM. Dental abnormalities in children treated for neuroblastoma. *Med Pediatr Oncol*. 1998;30(1):22–27. doi:10.1002/(sici)1096-911x(199801)30:1<22::aid-mpo8>3.0.co;2-2
14. Kang CM, Hahn SM, Kim HS, et al. Clinical risk factors influencing dental developmental disturbances in childhood cancer survivors. *Cancer Res Treat*. 2018;50(3):926–935. doi:10.4143/crt.2017.296
15. Wilberg P, Kanellopoulos A, Ruud E, Hjermstad MJ, Fossa SD, Herlofson BB. Dental abnormalities after chemotherapy in long-term survivors of childhood acute lymphoblastic leukemia 7–40 years after diagnosis. *Support Care Cancer*. 2016;24(4):1497–1506. doi:10.1007/s00520-015-2940-1
16. Gawade PL, Hudson MM, Kaste SC, et al. A systematic review of dental late effects in survivors of childhood cancer. *Pediatr Blood Cancer*. 2014;61(3):407–416. doi:10.1002/pbc.24842
17. Pedersen LB, Clausen N, Schrøder H, Schmidt M, Poulsen S. Microdontia and hypodontia of premolars and permanent molars in childhood cancer survivors after chemotherapy. *Int J Paediatr Dent*. 2012;22(4):239–243. doi:10.1111/j.1365-263X.2011.01199.x
18. Hölttä P, Alaluusua S, Saarinen-Pihkala UM, Peltola J, Hovi L. Agenesis and microdontia of permanent teeth as late adverse effects after stem cell transplantation in young children. *Cancer*. 2005;103(1):181–190. doi:10.1002/cncr.20762

19. Maguire A, Craft AW, Evans RG, et al. The long-term effects of treatment on the dental condition of children surviving malignant disease. *Cancer*. 1987;60(10):2570–2575. doi:10.1002/1097-0142(19871115)60:10<2570::AID-CNCR2820601037>3.0.CO;2-Q
20. Krasuska-Sławińska E, Brożyna A, Dembowska-Bagińska B, Olczak-Kowalczyk D. Antineoplastic chemotherapy and congenital tooth abnormalities in children and adolescents. *Contemp Oncol (Pozn)*. 2016;20(5):394–401. doi:10.5114/wo.2016.64602
21. Kaste SC, Goodman P, Leisenring W, et al. Impact of radiation and chemotherapy on risk of dental abnormalities: A report from the childhood cancer survivor study. *Cancer*. 2009;115(24):5817–5827. doi:10.1002/cncr.24670
22. Kinirons MJ, Fleming P, Boyd D. Dental caries experience of children in remission from acute lymphoblastic leukemia in relation to the duration of treatment and the period of time in remission. *Int J Paediatr Dent*. 1995;5(3):169–172. doi:10.1111/j.1365-263x.1995.tb00301.x
23. Näsman M, Forsberg CM, Dahllöf G. Long-term dental development in children after treatment for malignant disease. *Eur J Orthod*. 1997;19(2):151–159. doi:10.1093/ejo/19.2.151
24. Takuma S, Sawada T, Yama S, Yanagisawa T. Ultrastructural changes in the cementoblasts of rat molars after injection of vincristine. *J Dent Res*. 1984;63(9):1108–1115. doi:10.1177/00220345840630090201
25. Jodłowska A, Sobol-Milejska G, Postek-Stefańska L. A critical look at prevalence assessment of dental abnormalities after chemotherapy. Clinical research. *J Stoma*. 2019;72(3):95–105. doi:10.5114/jos.2019.87522
26. Jodłowska A, Pająk J, Postek-Stefańska L. Microdontia after chemotherapy in a patient treated for neuroblastoma: Histopathological findings. *Dent Med Probl*. 2018;55(3):343–349. doi:10.17219/dmp/95028
27. Krasuska-Sławińska E, Dembowska-Bagińska E, Brożyna A, Olczak-Kowalczyk D, Czarnowska E, Sowińska A. Changes in the chemical composition of mineralised teeth of children after antineoplastic treatment. *Contemp Oncol (Pozn)*. 2018;22(1):37–41. doi:10.5114/wo.2018.74392
28. Macleod RI, Welbury RR, Soames JV. Effects of cytotoxic chemotherapy on dental development. *J R Soc Med*. 1987;80(4):207–209. PMID:3585885. PMCID:PMC1290759.
29. Hantrakul S, Klangkaew N, Kunakornsawat S, et al. Clinical pharmacokinetics and effects of vincristine sulfate in dogs with transmissible venereal tumor (TVT). *J Med Vet Sci*. 2014;76(12):1549–1553. doi:10.1292/jvms.14-0180
30. Yan Z, Zhu Z, Qian Z, et al. Pharmacokinetics characteristics of vincristine sulfate liposomes in patients with advanced solid tumors. *Acta Pharmacol Sin*. 2012;33(6):852–858. doi:10.1038/aps.2012.44
31. Ramirez DA, Collins KP, Aradi AE, Conger KA, Gustafson DL. Kinetics of cyclophosphamide metabolism in humans, dogs, cats and mice and relationship to cytotoxic activity and pharmacokinetics. *Drug Metab Dispos*. 2019;47(3):257–268. doi:10.1124/dmd.118.083766
32. Hsieh SGS, Hibbert S, Shaw P, Ahern V, Arora M. Association of cyclophosphamide use with dental developmental defects and salivary gland dysfunction in recipients of childhood antineoplastic therapy. *Cancer*. 2011;117(10):2219–2227. doi:10.1002/cncr.25704

IRF6 rs2235371 as a risk factor for non-syndromic cleft palate only among the Deutero-Malay race in Indonesia and its effect on the *IRF6* mRNA expression level

Saskia Lenggogeni Nasroen[1,A–D,F], Ani Melani Maskoen[2,A,E,F], Hardisiswo Soedjana[3,C,E,F], Dany Hilmanto[4,C,E,F], Basri Abdul Gani[5,C–F]

[1] Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, the Jenderal Achmad Yani University, Cimahi, Bandung, Indonesia
[2] Department of Oral Biology, Faculty of Dentistry, Padjadjaran University, Bandung, Indonesia
[3] Division of Plastic Surgery Reconstruction and Esthetics, Department of Surgery, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia
[4] Department of Pediatrics, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia
[5] Department of Oral Biology, Faculty of Dentistry, Syiah Kuala University, Banda Aceh, Indonesia

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):59–65

Address for correspondence
Saskia Lenggogeni Nasroen
E-mail: saskiyaunjani@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
The authors are grateful to the Indonesian Cleft Lip and Palate Foundation and the Indonesian Cleft Center (Yayasan Pembina Penderita Celah Bibir dan Langit-langit – YPPCBL) in Bandung, Indonesia.

Received on June 5, 2021
Reviewed on September 10, 2021
Accepted on October 1, 2021

Published online on February 25, 2022

Cite as
Nasroen SL, Maskoen AM, Soedjana H, Hilmanto D, Gani BA. *IRF6* rs2235371 as a risk factor for non-syndromic cleft palate only among the Deutero-Malay race in Indonesia and its effect on the *IRF6* mRNA expression level. *Dent Med Probl.* 2022;59(1):59–65. doi:10.17219/dmp/142760

DOI
10.17219/dmp/142760

Abstract

Background. During the early embryological development of the face, complex orofacial failure results in a non-syndromic cleft lip and palate (NS CLP). The interferon regulatory factor 6 gene (*IRF6*) rs2235371 is a non-synonymous polymorphism that is one of the strong candidate genes associated with NS CLP.

Objectives. The purpose of this study was to determine *IRF6* rs2235371 as a risk factor for NS CLP and its phenotypes, including complete unilateral cleft lip and palate (CUCLP), bilateral cleft lip and palate (BCLP), cleft lip only (CL), and cleft palate only (CP), as well as to examine the effect of the polymorphism on the *IRF6* mRNA expression levels among the Deutero-Malay race in Indonesia.

Material and methods. This study used a case–control design and enrolled 264 samples, including 158 NS CLP cases (42 NS CUCLP, 34 NS BCLP, 33 NS CL, and 49 NS CP) and 106 control subjects. DNA was extracted from venous blood, and then subjected to polymerase chain reaction (PCR) and sequencing. The odds ratio (*OR*) was used to determine the risk factor for NS CLP and its phenotypes. The Livak, Kruskal–Wallis and Mann–Whitney *U* tests were used to determine mRNA expression levels in the oral epithelium, followed by real-time quantitative PCR (RT-qPCR).

Results. Among all of the NS CLP cases, in the NS CP phenotype, *OR* for the A mutant allele and the GA genotype was 2,492 ($p = 0.017$) and 2,114 ($p = 0.048$), respectively. The *IRF6* mRNA expression level of the GA genotype was higher in the NS CP subjects as compared to the GG genotype ($p = 0.031$).

Conclusions. The *IRF6* rs2235371 polymorphism is associated with the NS CP phenotype in Deutero-Malay patients from Indonesia and it affects the *IRF6* mRNA expression level.

Keywords: cleft lip and palate, cleft palate, *IRF6*, rs2235371, *IRF6* mRNA expression level

Introduction

Non-syndromic cleft lip and palate (NS CLP) is caused by the inability of any of the independently formed facial primordia of the orofacial complex to fuse completely during the early embryological development of the face.[1] It is a common congenital defect with a prevalence that varies according to the geographic region, the ethnic origin and the socioeconomic position.[2] The etiology of NS CLP is thought to be multifactorial, with several genetic and environmental factors combined, among which genetic factors need to be primarily analyzed. Non-syndromic cleft lip and palate exhibits complex phenotypes and is indicative of a breakdown in the standard mechanisms of the embryological process.[3] Cleft lip and palate cases are frequently classified into 2 groups, based on anatomical, genetic and embryological findings: those that involve the lip or the palate (CL/P) and those that involve only the palate (CP). Clefts are classified as syndromic or non-syndromic, based on the presence or absence of other physical or developmental anomalies in the affected individuals.[4]

The distinct embryological and pathophysiological mechanisms underlying NS CLP have also been reflected in classifying the condition into primary and secondary palate clefts. The primary palate comprises the maxillary lip, the alveolar process and the hard palate anterior to the incisive foramen.[5] The secondary palate is formed of tissues located posterior to the incisive foramen and extending to the hard and soft palates. Molecular evidence supports etiologic distinctions between the CLP subtypes – cleft lip only (CP) and cleft palate only (CP) – as well as the NS CLP phenotypes.[1]

Cleft lip only and CLP are not included in the CP mechanism, as there is compelling evidence that they are developmentally and genetically distinct from CP in most cases. They should be investigated independently and phenotyping is supposed to shed light on the previously unknown relationships.[6] Additionally, clefts can affect one or both halves of the oral cavity, and are thus classified as unilateral or bilateral clefts.[7] Bilateral cleft lip and palate (BCLP) is the most severe form of NS CLP and is thought to follow a genetic pattern that is distinct from other forms of NS CLP. Being one of the most prevalent anomalies, NS CLP is caused by genetic and environmental factors, neither of which has been fully elucidated due to the complex etiology of the disease.[8]

Several candidate genes are associated with an increased risk of NS CLP, including *RARA*, *PAX9*, *MTHFR*, *MSX1*, *BMP4*, *TGFB3*, *TGFA*, and *IRF6*. Interferon regulatory factor 6 (IRF6) is a transcription factor; a mutation in the gene *IRF6* is also known to cause van der Woude syndrome, which is the autosomal dominant condition of CLP with lip pits. *IRF6* is located on 1q32.2, with a highly conserved N-terminal DNA-binding domain (DBD) and a less conserved C-terminal Smad/IRF (SMIR)/interferon association domain (IAD) that has been associated with NS CLP in some populations through *IRF6* polymorphisms.[9] *IRF6* rs2235371 (V274I) is a single nucleotide polymorphism (SNP) in which valine is replaced by isoleucine at position 274 (exon 7) in the *IRF6* SMIR/IAD. It is associated with NS CLP in various populations.[10] Valine found at this location in *IRF6* is highly conserved across species. Previous research hypothesized that *IRF6* rs2235371 might affect the gene function and contribute to NS CLP.[11]

The *IRF6* rs2235371 polymorphism was the first marker in *IRF6* to be associated with NS CLP, most notably in the Asian (particularly East Asian) and South American populations, but not in Europeans, simply because the allele encoding isoleucine is absent or extremely rare in European people, with a maximum frequency of only 2%.[11] In genomic surveys, it was discovered that the polymorphisms affecting transcription and mRNA processing played a significant role in human phenotypic diversity and disease susceptibility. However, the majority of these polymorphisms remain unknown.[12]

We studied the influence of the *IRF6* rs2235371 polymorphism in patients with NS CLP of various phenotypes, such as complete unilateral cleft lip and palate (CUCLP), BCLP, CL, and CP, as well as in control subjects among Indonesians of the Deutero-Malay race. *IRF6* rs2235371 is a non-synonymous polymorphism that alters the amino acid (valine into isoleucine) functionally. A change in the mRNA conformation may affect the protein structure, activity and function, causing disease.[13] Understanding the impact of the polymorphism on the gene function may help discover the causal genes responsible for the phenotypes of NS CLP and other human diseases.

Material and methods

The Faculty of Medicine of Padjadjaran University, Bandung, Indonesia, has granted ethical clearance under ref. No. 395/UN6/C.1.2.3/KEPK/PN/2016. The entire sample was Deutero-Malay, as it is the major race in Indonesia. Only individuals diagnosed with a non-syndromic form of CLP were included in this study. A total of 246 subjects were enrolled; there were 158 NS CLP cases of various phenotypes (NS CUCLP – 42, NS BCLP – 34, NS CL – 33, and NS CP – 49) and 106 healthy controls. The study was conducted at the Molecular Genetic Laboratory of Padjadjaran University/Hasan Sadikin Hospital in Bandung, Indonesia. The subjects were divided into NS CUCLP and NS BCLP groups, as the authors assumed that since they presented different laterality and disease severity, they might have different genetic backgrounds. One of the hypotheses used to explain variable phenotypes in genetic disorders is frequently referred to as a genotype–phenotype correlation, in which the particular genotype is associated with the particular phenotype or the cleft side.[14]

Genotyping assay

DNA was extracted from each subject's venous blood by using DNA extraction kits (Sigma-Aldrich/Merck, Darmstadt, Germany). After obtaining written informed consent, venous blood samples were collected, DNA was extracted and the *IRF6* rs2235371 segment was amplified using polymerase chain reaction (PCR). The PCR analysis was performed using the primers of forward: 5'-CAGGGCTGCCGACTCTTCTA-3' and reverse: 5'-AGGAAAGCAGGAAGGTGAAAGA-3'.[15] The Sanger dideoxy method was used to perform DNA sequencing on *IRF6* rs2235371 in comparison with a standard nucleotide. All nucleotides in those segments were compared to a standard nucleotide in the gene bank, using a sequence alignment program from BioEdit, v. 7.2 (https://bioedit.software.informer.com/).[16] The polymorphism occurs when base G is replaced with A, resulting in 3 genotypes: GG (wild monozygous genotype); GA (heterozygous mutated genotype); and AA (homozygous mutated genotype).

RT-qPCR

After written informed consent was obtained from all subjects, oral epithelial cells from the cleft side were gathered from the NS CP subjects. For control subjects, oral epithelial cells were gathered from the palatal epithelium. All epithelial cells were collected using the smear method, and then stored in small tubes filled with RNA. Total RNA was extracted from those palatal epithelial cells with the TRIzol® reagent (Invitrogen, Waltham, USA) and the concentration was determined using the NanoDrop™ 2000c spectrophotometer (Thermo Scientific, Waltham, USA). Total RNA samples with adequate purity ratios (A260/A280: 1.9–2.1) were then processed with real-time quantitative PCR (RT-qPCR).[17] RNA was converted into cDNA by using an oligo (dT) primer and SuperScript II™ Reverse Transcriptase (Invitrogen). Internally, *GAPDH* was used as a reference gene. The relative mRNA expression of *IRF6* was quantified using RT-qPCR with the SYBR Green™ method (SensiFAST™ SYBR; Bioline USA, Memphis, USA). The primers for *IRF6* were 5'-CGGCATAGCCCTCAACAAGAA-3' and 5'-TCCTTGGTGCCATCATACATCAG-3', and for *GAPDH*, they were 5'-TGCTGAGTATGTCGTGGAG-3' and 5'-GTCTTCTGAGTGGCAGTGAT-3'.[15]

Statistical analysis

Significant differences in the frequency of sequence variants between NS CLP subjects, various NS CLP phenotypes (NS CUCLP, NS BCLP, NS CL, and NS CP) and control subjects were determined using the statistical analysis. The odds ratio (*OR*) was used to identify the risk factor for NS CLP and its phenotypes. Differences in the *IRF6* mRNA expression between the NS CP patients with various genotypes (GG, GA and AA) and controls were then analyzed using the Livak method. Changes in the *IRF6* mRNA expression were determined using the comparative CT method ($2^{-\Delta\Delta Ct}$). The statistical analysis involved the Kruskal–Wallis test, followed by the Mann–Whitney *U* test to compare the groups. The IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA) was used, with a p-value <0.05 considered as statistically significant.

Results

Figure 1 presents the initial PCR product that showed a DNA band of 413 base pairs (bp). After obtaining the initial PCR products of 413 bp, the samples were analyzed with the Sanger dideoxy sequencing method. The sequencing results for all subjects showed the GG, GA and AA genotypes (Fig. 2). Figure 3 shows that among the NC CP subjects, the GA heterozygous mutated genotype presented a higher level of the *IRF6* mRNA expression (overexpression) (36.41 ±85.70) as compared to the GG genotype (3.77 ±23.76) (p = 0.031).

There were no statistically significant differences between the alleles and the genotypes in terms of an increased risk of the disease in NS CLP (in total), NS CUCLP and NS BCLP subjects as compared to controls (Tables 1–3). *IRF6* rs2235371 was not a risk factor for NS CLP in general. *IRF6* rs2235371 was also analyzed

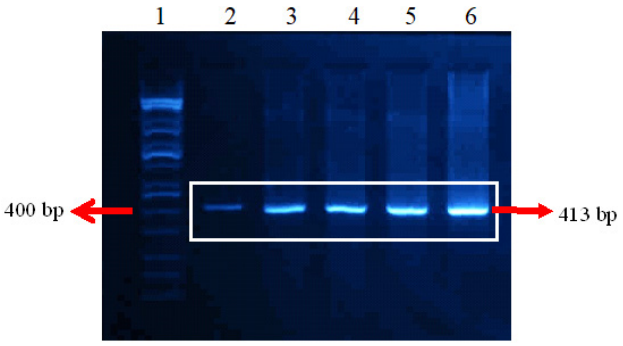

Fig. 1. Initial polymerase chain reaction (PCR) product of the *IRF6* rs2235371 segment

Line 1 – 100 base pairs (bp) ladder; lines 2–6 – initial PCR product.

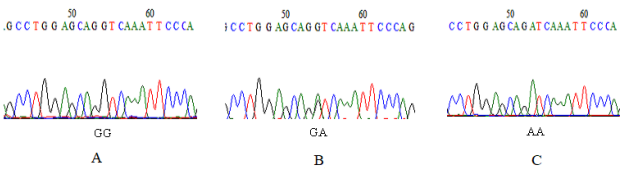

Fig. 2. Sequencing results showing 3 genotypes

A – GG genotype; B – GA genotype; C – AA genotype.

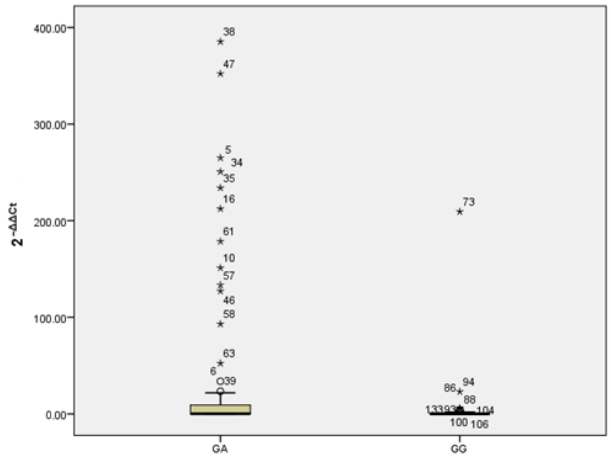

Fig. 3. Significant association between the GA and GG genotypes of the non-syndromic cleft palate only (NS CP) group and the *IRF6* mRNA expression ($2^{-\Delta\Delta Ct}$); the GA genotype shows significant mRNA overexpression as compared to the GG genotype

in the NS CUCLP phenotype and it turned out that *IRF6* rs2235371 was not a risk factor for NS CUCLP. As far as the NS BCLP phenotype is concerned, which is the most severe type of NS CLP, *IRF6* rs2235371 proved not to be a risk factor for NS BCLP as well. Table 4 shows that *IRF6* rs2235371 was not a risk factor for the NS CL phenotype as the most straightforward type of NS CLP. The AA genotype was not found in the NS CL phenotype subjects. However, in the case of the NS CP phenotype, it was shown that *IRF6* rs2235371 was a risk factor for NS CP, especially in the presence of the A mutant allele and the GA heterozygous mutated genotype, as the results were statistically significant (Table 5). Table 6 shows the influence of the GA genotype on the *IRF6* mRNA expression level among the NS CP subjects. The epithelial cells were taken from the oral mucosa, and then evaluated by means of RT-qPCR. The GA heterozygous mutated genotype as a risk factor for NS CP showed a statistically significant difference in terms of *IRF6* mRNA expression as compared to the normal GG genotype (Table 6). We did not analyze the *IRF6* mRNA expression levels for the AA genotype, since this genotype did not prove to be the risk factor associated with NS CP. Also, as *IRF6* rs2235371 was a risk factor for NS CP, especially in the case of the A allele and the GA genotype, we only compared the *IRF6* mRNA expression in the NS CP phenotype with the controls.

Table 1. Allele and genotype frequency of nucleotides G and A from *IRF6* rs2235371 in the non-syndromic cleft lip and palate (NS CLP) (in total) subjects

Allele/Genotype	NS CLP *n* (%)	Control *n* (%)	χ^2	*p*-value	*OR*	95% *CI*
G	229 (72.47)	157 (74.06)	0.000	1.000	1.046	0.385–2.841
A	87 (27.53)	55 (25.94)	0.180	0.671	1.149	0.701–1.882
GG	81 (51.27)	58 (54.72)	0.180	0.671	0.871	0.531–1.426
GA	67 (42.41)	41 (38.68)	0.226	0.634	1.167	0.706–1.929
AA	10 (6.33)	7 (6.60)	0.000	1.000	0.956	0.352–2.595

n – number; *OR* – odds ratio; *CI* – confidence interval; G – wild type allele; A – mutant allele; GG – wild monozygous genotype; GA – heterozygous mutated genotype; AA – homozygous mutated genotype.

Table 2. Allele and genotype frequency of nucleotides G and A from *IRF6* rs2235371 in the non-syndromic complete unilateral cleft lip and palate (NS CUCLP) subjects

Allele/Genotype	NS CUCLP *n* (%)	Control *n* (%)	χ^2	*p*-value	*OR*	95% *CI*
G	56 (66.67)	157 (74.06)	0.000	1.000	0.919	0.226–3.736
A	28 (33.33)	55 (25.94)	1.904	0.168	1.777	0.860–3.670
GG	17 (40.48)	58 (54.72)	1.904	0.168	0.563	0.273–1.162
GA	22 (52.38)	41 (38.68)	1.784	0.182	1.744	0.848–3.585
AA	3 (7.14)	7 (6.60)	0.000	1.000	1.088	0.268–4.422

Table 3. Allele and genotype frequency of nucleotides G and A from *IRF6* rs2235371 in the non-syndromic bilateral cleft lip and palate (NS BCLP) subjects

Allele/Genotype	NS BCLP *n* (%)	Control *n* (%)	χ^2	*p*-value	*OR*	95% *CI*
G	56 (82.35)	157 (74.06)	0.000	1.000	1.131	0.224–5.724
A	12 (17.65)	55 (25.94)	2.058	0.151	0.503	0.219–1.156
GG	24 (70.59)	58 (54.72)	2.058	0.151	1.986	0.865–4.559
GA	8 (23.53)	41 (38.68)	1.974	0.160	0.488	0.202–1.180
AA	2 (5.88)	7 (6.60)	0.000	1.000	0.884	0.175–4.472

Table 4. Allele and genotype frequency of nucleotides G and A from *IRF6* rs2235371 in the non-syndromic cleft lip only (NS CL) subjects

Allele/Genotype	NS CL *n* (%)	Control *n* (%)	χ^2	*p*-value	*OR*	95% *CI*
G	57 (86.36)	157 (74.06)	1.122	0.290	–	–
A	9 (13.64)	55 (25.94)	2.671	0.102	0.453	0.192–1.067
GG	24 (72.73)	58 (54.72)	2.671	0.102	2.207	0.937–5.196
GA	9 (27.27)	41 (38.68)	0.969	0.325	0.595	0.252–1.405
AA	–	7 (6.60)	1.122	0.290	–	–

Table 5. Allele and genotype frequency of nucleotides G and A from *IRF6* rs2235371 in the non-syndromic cleft palate only (NS CP) subjects

Allele/Genotype	NS CP *n* (%)	Control *n* (%)	χ^2	*p*-value	*OR*	95% *CI*
G	60 (61.22)	157 (74.06)	0.209	0.648	0.622	0.187–2.069
A	38 (38.78)	55 (25.94)	5.684	0.017*	2.492	1.226–5.064
GG	16 (32.65)	58 (54.72)	5.684	0.017*	0.401	0.197–0.815
GA	28 (57.14)	41 (38.68)	3.908	0.048*	2.114	1.063–4.205
AA	5 (10.20)	7 (6.60)	0.209	0.648	1.607	0.483–5.343

* statistically significant.

Table 6. Comparison of the *IRF6* mRNA expression between the GA and GG genotypes from the non-syndromic cleft palate only (NS CP) group ($2^{-\Delta\Delta Ct}$)

Genotype	*M* ±*SD*	*Me*	Range	*p*-value
GA	36.42 ±85.70	0.27	0.00–385.34	0.031*
GG	3.77 ±23.79	0.08	0.00–209.38	

M – mean; *SD* – standard deviation; *Me* – median; * statistically significant.

Tables 5,6 and Fig. 3 report only the NS CP subjects to present significant results in the case of the A allele (*OR*: 2.492; 95% confidence interval (*CI*): 1.226–5.064; $p = 0.017$) and the GA genotype (*OR*: 2.114; 95% *CI*: 1.063–4.205); $p = 0.048$). The subjects with NS CP and the controls who were carrying the GA genotype presented a higher level of the *IRF6* mRNA expression (overexpression) (36.41 ±85.70) as compared to the subjects with the GG genotype (3.77 ±23.76) ($p = 0.031$)

The allelic frequency of the G normal allele and the A mutant allele, and the frequency of the GG, GA and AA genotypes among the NS CLP subjects, as well as for each phenotype (NS CUCLP, NS BCLP, NS CL, and NS CP), and control subjects were compared using the χ^2 test (Tables 1–5).

Discussion

The *IRF6* gene is located on chromosome 1q32.2 and consists of 9 exons. It is involved in epidermal differentiation, as mice lacking *IRF6* have a hyperproliferative epidermis that fails to differentiate terminally. *IRF6* controls the transition between proliferation and differentiation. The loss of the gene function or a mutation/polymorphism results in the incomplete fusion of palatal shelves due to failure in the differentiation of the palatal medial edge epithelial (MEE) cells through the epithelial–mesenchymal transformation (EMT).[18]

IRF6 rs2235371 is a polymorphism in the exon region caused by exonic changes that are translated into protein. Polymorphisms in the exon region can be either non-synonymous (protein-changing) or synonymous (non-protein-changing). A non-synonymous polymorphism alters the amino acid sequence within the protein structure and affects the normal protein function as a transcription factor. We assumed that *IRF6* rs2235371 would change protein conformation and disrupt the gene functionally. Hence, failure in the terminal differentiation of MEE cells would occur, leading to an undifferentiated hyperproliferative epidermis.[19]

Numerous studies have demonstrated that *IRF6* polymorphisms may play a significant role in CL, implying that patients with CL may be more susceptible to genetic changes in *IRF6* than patients with CLP or CP. Additionally, this evidence supports the hypothesis that CL is genetically and developmentally distinct from CLP and CP.[1] This finding contrasts with our present study, which found no significant association between *IRF6* rs2235371 and CL. In contrast to some previous studies, our current study discovered a significant association between *IRF6* rs2235371 and CP. Previous studies have not completely defined the association of *IRF6* rs2235371 with NS CLP phenotypes.

The rs2235371 in *IRF6* is a missense mutation in the protein binding domain (PBD) or SMIR/IAD of *IRF6*. In contrast to what has been observed in the African and European populations, this SNP is highly polymorphic in the Asian population. However, since it is located in PBD or SMIR/IAD, the function of *IRF6* rs2235371 cannot be fully explained.[20] According to a previous study, only one mutation, p.Phe375Ser, is located in the core of PBD and significantly affects the domain folding and function.[21] The transition

from a more considerable hydrophilic residue to a minor hydrophilic residue eliminates many of the stabilizing hydrophobic interactions of the phenylalanine side chain.[21]

In Japan, China, Vietnam, Philippines, South America, and Iran, but not in Europe or India, *IRF6* rs2235371 has been observed to be significantly associated with NS CLP.[22] A study conducted by Park et al. revealed that while *IRF6* rs2235371 was not a causal polymorphism for NS CLP, it appeared to interact and be in linkage disequilibrium (LD) with other *IRF6* polymorphisms that were causal polymorphisms for NS CLP.[10] This was also observed in the Mexican population.[10] A study by Tang et al. revealed that the association between *IRF6* rs2235371 and NS CLP had been extensively studied in Chinese people, with mixed results.[23] Similar to our findings, the A allele of *IRF6* rs2235371 might increase the risk of developing CP in Chinese subjects.[23] In comparison, another study conducted in China discovered that the A allele was more prevalent in controls and appeared to be protective according to the haplotype analysis; this type of analysis is usually used to investigate various population phenomena, including migration and immigration rates, the strength of LD, and population relatedness.[24] Zucchero et al. found that the carriers of the G allele (Val274) of *IRF6* rs2235371 were at higher risk of the recurrence of NS CLP.[25] They established strong evidence for excessive valine allele transmission from the parent to the affected child in CLP families.[25] In comparison, Suazo et al. reported that the C allele of rs2235375 from the *IRF6* gene seemed to be a risk factor for NS CLP in the Chilean population.[26] Furthermore, a genetic variant of *IRF6* might be a protective factor against NS CLP, while the *Rsa1* gene variant (the A allele) could be considered the risk factor associated with the development of NS CLP.[27] Individuals heterozygous for this specific polymorphism (the GA genotype) are at lower risk of the recurrence of CLP than those who are homozygous for the G allele (the GG genotype).[27] The disparate findings may result from diverse ethnic origins, environmental differences, anthropological diversity, different research methods, and the complex genetic etiology of the disease.[28]

According to our present findings, *IRF6* rs2235371 appears to play a functional role in the development of the secondary palate, as it was a risk factor for NS CP in both the A allele (*OR*: 2.492; 95% *CI*: 1.226–5.064); $p = 0.017$) and the GA genotype (*OR*: 2.114; 95% *CI*: 1.063–4.205; $p = 0.048$) groups. However, since the precise biochemical role of *IRF6* rs2235371 in the etiology of NS CP is unknown, additional experimental studies are necessary to establish a direct functional link between *IRF6* and NS CP. *IRF6* mutant mice develop a hyperproliferative epidermis that fails to differentiate terminally, resulting in incomplete soft tissue fusion, indicating that *IRF6* is the critical determinant of the keratinocyte proliferation–differentiation switch.[29] Thus, future experiments may focus on the role of the *IRF6* exon region as a coding region in the proliferation–differentiation switch of keratinocytes during the development of the secondary palate, and whether such exonic changes cause protein damage or dysfunction. Mutations in the *IRF6* gene have been identified as the cause of van der Woude syndrome and mutations in the poliovirus receptor-related 1 gene (*PVRL1*) have been identified as the cause of autosomal recessive ectodermal dysplasia syndrome, which is associated with clefting.[30]

The level of mRNA expression can be considered an intermediate phenotype, and correlating the polymorphism and the mRNA expression level is critical for elucidating the physiological traits associated with human diseases.[31] Polymorphisms in coding genes, particularly non-synonymous polymorphisms, such as *IRF6* rs2235371, can significantly affect protein regulation as compared to non-coding genes, as they alter the structure and function of the resulting peptide due to the altered chemical and physical properties of the altered forms of mRNA secondary structure. However, the effect of non-synonymous polymorphisms on protein expression via mRNA structural modification is essentially unknown.[32]

The *IRF6* rs2235371 (c.820G>A) polymorphism in the exon region may alter the amino acid sequence translated into the protein structure, and thus affect the normal function of the transcription factor.[33] Such exonic changes may also influence mRNA stability, thereby affecting the translational efficiency of the gene. However, the functional significance of *IRF6* rs2235371, which considerably affects the *IRF6* mRNA expression level when associated with the NS CP phenotype, has not been defined. We assumed that *IRF6* rs2235371 would change protein conformation and disrupt the gene functionally, which was confirmed in this study by the observed overexpression of the gene. Thus, MEE cells could not reach terminal differentiation, which resulted in an undifferentiated hyperproliferative epidermis.

Conclusions

The *IRF6* gene variant rs2235371 is considered a risk factor for the NS CP phenotype in the Deutero-Malay race of Indonesian patients. The *IRF6* mRNA expression level in NS CP and control cells reveals that the GA genotype is overexpressed ($p = 0.031$). It demonstrates that the GA genotype may induce *IRF6* mRNA expression alterations. A further genome-wide approach, such as exclusive exon screening in the *IRF6* gene, could be an excellent way to follow up on the findings of the present study, particularly in defining the precise role of this gene in NC CP pathogenesis.

Ethics approval and consent to participate

The study was approved by the institutional Research Ethics Committee (No. of approval 395/UN6/C.1.2.3/KEPK/PN/2016) and all participants provided written informed consent prior to the investigation.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Saskia Lenggogeni Nasroen https://orcid.org/0000-0002-9838-9899
Ani Melani Maskoen https://orcid.org/0000-0001-7707-0582
Hardisiswo Soedjana https://orcid.org/0000-0001-5367-463X
Dany Hilmanto https://orcid.org/0000-0003-3684-4144
Basri Abdul Gani https://orcid.org/0000-0003-2438-1226

References

1. Rahimov F, Jugessur A, Murray JC. Genetics of nonsyndromic orofacial clefts. *Cleft Palate Craniofac J*. 2012;49(1):73–91. doi:10.1597/10-178
2. Impellizzeri A, Giannantoni I, Polimeni A, Barbato E, Galluccio G. Epidemiological characteristic of orofacial clefts and its associated congenital anomalies: Retrospective study. *BMC Oral Health*. 2019;19(1):290. doi:10.1186/s12903-019-0980-5
3. Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: Understanding genetic and environmental influences. *Nat Rev Genet*. 2011;12(3):167–178. doi:10.1038/nrg2933
4. Curtis SW, Chang D, Lee MK, et al. The *PAX1* locus at 20p11 is a potential genetic modifier for bilateral cleft lip. *HGG Adv*. 2021;2(2):100025. doi:10.1016/j.xhgg.2021.100025
5. Kosowski TR, Weathers WM, Wolfswinkel EM, Ridgway EB. Cleft palate. *Semin Plast Surg*. 2012;26(4):164–169. doi:10.1055/s-0033-1333883
6. Huang L, Jia Z, Shi Y, et al. Genetic factors define CPO and CLO subtypes of nonsyndromicorofacial cleft. *PLoS Genet*. 2019;15(10):e1008357. doi:10.1371/journal.pgen.1008357
7. de Souza Freitas JA, das Neves LT, Fraga de Almeida AL, et al. Rehabilitative treatment of cleft lip and palate: Experience of the Hospital for Rehabilitation of Craniofacial Anomalies/USP (HRAC/USP) – part 1: overall aspects. *J Appl Oral Sci*. 2012;20(1):9–15. doi:10.1590/s1678-77572012000100003
8. Cobourne MT. The complex genetics of cleft lip and palate. *Eur J Orthod*. 2004;26(1):7–16. doi:10.1093/ejo/26.1.7
9. Zhang M, Huang J, Shi F, et al. Identification of pathogenic variant in a Chinese pedigree affected with non-syndromic cleft lip and palate [in Chinese]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*. 2021;38(1):52–55. doi:10.3760/cma.j.cn511374-20200308-00144
10. Park JW, McIntosh I, Hetmanski JB, et al. Association between *IRF6* and non-syndromic cleft lip with or without cleft palate in four populations. *Genet Med*. 2007;9(4):219–227. doi:10.1097/gim.0b013e3180423cca
11. Birnbaum S, Ludwig KU, Reutter H, et al. *IRF6* gene variants in Central European patients with non-syndromic cleft lip with or without cleft palate. *Eur J Oral Sci*. 2009;117(6):766–769. doi:10.1111/j.1600-0722.2009.00680.x
12. Manning KS, Cooper TA. The roles of RNA processing in translating genotype to phenotype. *Nat Rev Mol Cell Biol*. 2017;18(2):102–114. doi:10.1038/nrm.2016.139
13. Zhang Z, Miteva MA, Wang L, Alexov E. Analyzing effects of naturally occurring missense mutations. *Comput Math Methods Med*. 2012;2012:805827. doi:10.1155/2012/805827
14. Goldstein I, Ehrenreich IM. The complex role of genetic background in shaping the effects of spontaneous and induced mutations. *Yeast*. 2021;38(3):187–196. doi:10.1002/yea.3530
15. Pan Y, Ma J, Zhang W, et al. *IRF6* polymorphisms are associated with non-syndromic orofacial clefts in a Chinese Han population. *Am J Med Genet A*. 2010;152A(10):2505–2511. doi:10.1002/ajmg.a.33624
16. Zerbino DR, Achuthan P, Akanni W, et al. Ensembl 2018. *Nucleic Acids Res*. 2018;46(D1):D754–D761. doi:10.1093/nar/gkx1098
17. Ma L, Li Z, Liu K, et al. Association of genetic variants with polypoidal choroidal vasculopathy: A systematic review and updated meta-analysis. *Ophthalmology*. 2015;122(9):1854–1865. doi:10.1016/j.ophtha.2015.05.012
18. Richardson RJ, Dixon J, Malhotra S, et al. *Irf6* is a key determinant of the keratinocyte proliferation–differentiation switch. *Nat Genet*. 2006;38(11):1329–1334. doi:10.1038/ng1894
19. Degen M, Girousi E, Feldmann J, et al. A novel Van der Woude syndrome-causing *IRF6* variant is subject to incomplete non-sense-mediated mRNA decay affecting the phenotype of keratinocytes. *Front Cell Dev Biol*. 2020;8:583115. doi:10.3389/fcell.2020.583115
20. Majewski J, Pastinen T. The study of eQTL variations by RNA-seq: from SNPs to phenotypes. *Trends in Genet*. 2011;27(2):72–79. doi:10.1016/j.tig.2010.10.006
21. Phillips DC, York RL, Mermut O, McCrea KR, Ward RS, Somorjai GA. Side chain, chain length, and sequence effects on amphiphilic peptide adsorption at hydrophobic and hydrophilic surfaces studied by sum-frequency generation vibrational spectroscopy and quartz crystal microbalance. *J Physic Chem C*. 2007;111(1):255–261. doi:10.1021/jp0645263
22. Xia Y, Hu B, Chen J, Zheng L, Song J. Association between the *IRF6* rs2235371 polymorphism and the risk of nonsyndromic cleft lip with or without cleft palate in Chinese Han populations: A meta-analysis. *Arch Oral Biol*. 2017;84:161–168. doi:10.1016/j.archoralbio.2017.09.032
23. Tang W, Du X, Feng F, et al. Association analysis between the *IRF6* G820A polymorphism and nonsyndromic cleft lip and/or cleft palate in a Chinese population. *Cleft Palate Craniofac J*. 2009;46(1):89–92. doi:10.1597/07-131.1
24. Fallin D, Schork NJ. Accuracy of haplotype frequency estimation for biallelic loci, via the expectation–maximization algorithm for unphased diploid genotype data. *Am J Hum Genet*. 2000;67(4):947–959. doi:10.1086/303069
25. Zucchero TM, Cooper ME, Maher BS, et al. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *N Engl J Med*. 2004;351(8):769–780. doi:10.1056/NEJMoa032909
26. Suazo J, Recabarren AS, Marín NR, Blanco R. Association between *IRF6* variants and nonsyndromic cleft lip with or without cleft palate in Chile. *Reprod Sci*. 2020;27(10):1857–1862. doi:10.1007/s43032-020-00203-9
27. Sufiawati I, Maskoen AM, Soeria Soemantri ES. Genetic variation of *IRF6* and *TGFA* genes in an HIV-exposed newborn with non-syndromic cleft lip palate. *Oral Dis*. 2020;26(Suppl 1):165–168. doi:10.1111/odi.13403
28. Mijiti A, Ling W, Guli, Moming A. Association of single-nucleotide polymorphisms in the *IRF6* gene with non-syndromic cleft lip with or without cleft palate in the Xinjiang Uyghur population. *Br J Oral Maxillofac Surg*. 2015;53(3):268–274. doi:10.1016/j.bjoms.2014.12.008
29. Pegelow M, Koillinen H, Magnusson M, et al. Association and mutation analyses of the *IRF6* gene in families with nonsyndromic and syndromic cleft lip and/or cleft palate. *Cleft Palate Craniofac J*. 2014;51(1):49–55. doi:10.1597/11-220
30. Cobourne MT. The complex genetics of cleft lip and palate. *Eur J Orthod*. 2004;26(1):7–16. doi:10.1093/ejo/26.1.7
31. Squires KE, Montañez-Miranda C, Pandya RR, Torres MP, Hepler JR. Genetic analysis of rare human variants of regulators of G protein signaling proteins and their role in human physiology and disease. *Pharmacol Rev*. 2018;70(3):446–474. doi:10.1124/pr.117.015354
32. Wang M, Pan Y, Zhang Z, Wang L. Three polymorphisms in *IRF6* and 8q24 are associated with nonsyndromic cleft lip with or without cleft palate: Evidence from 20 studies. *Am J Med Genet A*. 2012;158A(12):3080–3086. doi:10.1002/ajmg.a.35634
33. Stergachis AB, Haugen E, Shafer A, et al. Exonic transcription factor binding directs codon choice and affects protein evolution. *Science*. 2013;342(6164):1367–1372. doi:10.1126/science.1243490

Effects of the *Hydnophytum formicarum* plant extract on collagen density, angiogenesis, wound length, and re-epithelialization in wound healing: Experimental study on rats

Nissia Ananda[1,B–D], Dwi Ariawan[2,A,E,F], Vetnizah Juniantito[3,A,C,E,F]

[1] Residency Program, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Indonesia, Jakarta, Indonesia
[2] Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Indonesia, Jakarta, Indonesia
[3] Department of Veterinary Clinic, Reproduction and Pathology, Faculty of Veterinary Medicine, IPB University (Institut Pertanian Bogor), Bogor, Indonesia

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):67–73

Address for correspondence
Dwi Ariawan
E-mail: dwiariawan75@gmail.com

Funding sources
This study was supported within the PUTI Saintekes 2020 program by the University of Indonesia, Jakarta, Indonesia (NKB-4827/UN2.RST/HKP.05.00/2020).

Conflict of interest
None declared

Acknowledgements
None declared

Received on May 17, 2021
Reviewed on July 4, 2021
Accepted on July 14, 2021

Published online on March 10, 2022

Cite as
Ananda N, Ariawan D, Juniantito V. Effects of the *Hydnophytum formicarum* plant extract on collagen density, angiogenesis, wound length, and re-epithelialization in wound healing: Experimental study on rats. *Dent Med Probl.* 2022;59(1):67–73. doi:10.17219/dmp/140208

DOI
10.17219/dmp/140208

Abstract

Background. The formation of scar tissue in the wound healing process is associated with fibroblasts that are produced during the proliferation phase (3–14 days after surgery/injury). One of the strategies to suppress the formation of excessive scar tissue is to use wound care material. The use of herbal extracts is currently being investigated by researchers, as it allows avoiding the side effects of synthetic drugs. The *Hydnophytum formicarum* extract has antioxidant and anti-inflammatory potential.

Objectives. The aim of the study was to analyze the effects of the *Hydnophytum formicarum* plant extract on collagen density, angiogenesis, wound length, and re-epithelialization in wound healing.

Material and methods. Twenty-four Sprague–Dawley rats were divided into 2 groups: the control group; and the treatment group. Skin wounds were made on the dorsum of the rats, using the biopsy punch technique. Four rats from each group were sacrificed on days 4, 7 and 14 after injury. Collagen density, angiogenesis, wound length, and re-epithelialization were analyzed using hematoxylin and eosin (H&E) staining and Masson's trichrome staining.

Results. There were significant differences in the results of the angiogenesis analysis, wound length and re-epithelialization between the treatment and control groups. When considering angiogenesis, there were fewer vessels in the treatment group, but they were more mature as compared to the control group. There was also a meaningful interaction between the application of the *Hydnophytum formicarum* extract and the necropsy day with regard to collagen density and the re-epithelialization rate. No secondary infection was found in either group.

Conclusions. The topical use of the *Hydnophytum formicarum* extract affected the formation of scar tissue, as indicated by the positive area of collagen, the extent of angiogenesis, wound length, and the re-epithelialization rate in the early, middle and final granulation phases. The inhibition of angiogenesis through the application of *Hydnophytum formicarum* was probably related to the formation of scar tissue in the wound.

Keywords: angiogenesis, wound healing, scar formation, *Hydnophytum formicarum*

Introduction

Oral and maxillofacial surgeons often treat patients with skin wounds; the occurrence of these wounds activates the wound healing process. Wound healing is a process of restoration of the anatomical and physiological integrity of the injured tissues that consists of several phases (hemostasis, inflammation, proliferation, and remodeling).[1,2] Scar tissue that forms during the wound healing process is indeed a common complication. Hypertrophic scar tissue results in skin deformity, malfunctioning and psychological distress.[3]

During the inflammation phase, macrophages release cytokines and growth factors that attract fibroblasts to the wounded area. The proliferation phase starts on the 3rd day after injury and is marked by the presence of fibroblasts.[1] Fibroblast activities include the production of collagen, the induction of angiogenesis and the contraction of wounds, as well as the facilitation of re-epithelialization, which leads to the formation of hypertrophic scar tissue if it happens excessively.[4]

Many strategies have been used to reduce excessive scar tissue, including the use of herbal agents.[5] People tend to use herbal medicines to avoid the side effects of synthetic drugs, chiefly in developing countries.[6] *Hydnophytum formicarum* is one of many medicinal plants known to Southeast Asian people. The tuber part of this plant is believed to cure various diseases. In addition, it is generally accepted that *Hydnophytum formicarum* has a significant anti-inflammatory effect that inhibits scar formation.[7,8] Thus, this study aimed to collect baseline data and analyze the effects of *Hydnophytum formicarum* on collagen density, angiogenesis, wound length, and the re-epithelialization rate as an indicator of scar formation in the early, middle and late proliferation phases.

Material and methods

This research was conducted at the Faculty of Medicine of the University of Indonesia, Jakarta, and the Faculty of Veterinary Medicine of the IPB University (Institut Pertanian Bogor), Bogor, Indonesia, from August until November 2020.

Hydnophytum formicarum extract

The *Hydnophytum formicarum* plants were cleaned, dried and macerated using ethanol. The separation of ethanol and the active compound of the extract was accomplished by applying the rotary evaporator technique. The density and percentage of the active compound were determined.

Rats

Twenty-four 8-week-old Sprague–Dawley rats with a body weight of 200–300 g were used in this study. The rats were randomly divided into 2 groups: the control group; and the treatment group. The rats were kept in accordance with the applicable standards in animal cages in the animal research facilities of the Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, University of Indonesia. The experiment was approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia (protocol No. 20-07-0783), as well as Dr. Cipto Mangunkusumo National Central Public Hospital, Jakarta, Indonesia.

Wound model

Anesthesia was performed with an intraperitoneal injection of a ketamine/xylazine solution. Circular, full-thickness skin wounds of a diameter of 8 mm were made in the dorsum of the rats with punch biopsy tools. Wound dressing changes were carried out every day and in the treatment group, the *Hydnophytum formicarum* extract was applied topically on the wounds. Four rats from each group were anesthetized and sacrificed on days 4, 7 and 14, using the exsanguination technique. No antibiotics or analgesics were used in this study.

Wound healing observation

The wound areas were resected and fixated in 10% formalin. Histopathological observations were carried out using hematoxylin and eosin (H&E) staining and Masson's trichrome staining. Quantitative analyses of collagen density, angiogenesis, wound length, and re-epithelialization were performed using the ImageJ software (https://imagej.nih.gov/ij/index.html).

The appearance of collagen density areas in Masson's trichrome staining is characterized by a bluish color in thick, wavy and having transverse fibers cytoplasm without the nucleus. The collagen density area [mm^2] was measured as the total area of collagen in relation to the total wound area in 6 regions. The image of angiogenesis which emerges from H&E staining is in the form of purple endothelial cells and red-colored erythrocytes. The total number of blood vessels found in the 6 regions was used as a measurement of angiogenesis. Wound length [mm] was determined by measuring the distance between both edges of the wound, based on the H&E staining examination. The re-epithelialization rate was calculated using the following equation (Equation 1):

$$\text{re-epithelialization rate} = \frac{S_t}{S_0} \times 100\% \quad (1)$$

where:
S_t – residual wound area at the indicated time; and
S_0 – initial wound area.

Statistical analysis

The data was statistically analyzed using the IBM SPSS Statistics for Windows software, v. 26.0 (IBM Corp., Armonk, USA). The normality of data distribution was tested using the Shapiro–Wilk test, and the parametric two-way analysis of variance (ANOVA) was performed to compare differences between the groups and the necropsy days.

Results

The values for collagen density, angiogenesis, wound length, and the re-epithelialization rate are presented in Table 1. Generally, the average values of collagen density and the mean re-epithelialization rates in both the control and treatment groups increased from the early to late proliferation phases, while wound length decreased. The average angiogenesis values in both the control and treatment groups peaked in the middle proliferation phase (Fig. 1).

The Shapiro–Wilk test showed normally distributed data ($p > 0.05$). The two-way ANOVA with Tukey's post hoc honestly significant difference (HSD) test was used to compare the wound healing variables (collagen density, angiogenesis, wound length, and the re-epithelialization rate) between the groups and the necropsy days, as presented in Table 2 and Table 3.

Table 1. Collagen density, angiogenesis, wound length, and the re-epithelialization rate in the study groups on different necropsy days

Variable	Control group			Treatment group		
	day 4	day 7	day 14	day 4	day 7	day 14
Collagen density area [mm^2]	0.04 ±0.03	0.10 ±0.05	0.29 ±0.33	0.04 ±0.01	0.13 ±0.07	0.19 ±0.02
Angiogenesis (*n*)	46.75 ±12.97	70.50 ±15.02	41.00 ±23.65	11.75 ±7.13	40.25 ±16.21	19.00 ±7.43
Wound length [mm]	9.66 ±2.62	6.24 ±3.02	2.18 ±4.23	12.73 ±4.96	9.62 ±2.36	7.27 ±2.83
Re-epithelization rate [%]	0.38 ±0.49	18.61 ±11.35	84.84 ±30.31	2.27 ±1.63	14.59 ±10.29	36.25 ±16.09

Data presented as mean ± standard deviation (*M* ±*SD*). *n* – total number of blood vessels.

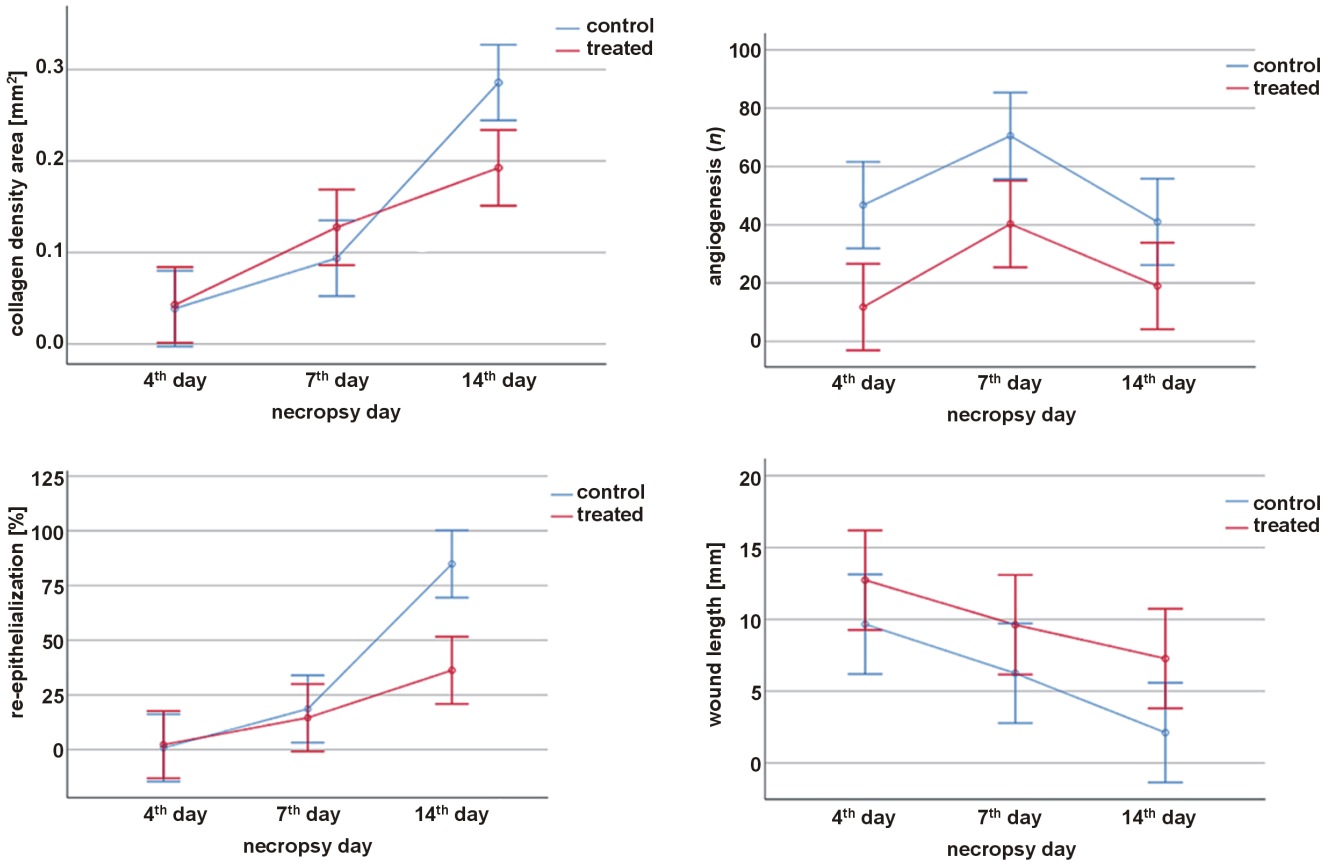

Fig. 1. Line graphs showing collagen density, angiogenesis, wound length, and the re-epithelialization rate in the study groups on different necropsy days

Table 2. Results of the two-way ANOVA with regard to differences in collagen density, angiogenesis, wound length, and the re-epithelialization rate between the groups and the associated necropsy days

Variable	Comparison		*F*	*p*-value
Collagen density	groups	control	1.201	0.288
		treatment		
	necropsy days	4	47.359	0.000*
		7		
		14		
	interaction test group–necropsy day		5.159	0.017*
Angiogenesis	groups	control	23.026	0.000*
		treatment		
	necropsy days	4	8.028	0.003*
		7		
		14		
	interaction test group–necropsy day		0.393	0.681
Wound length	groups	control	7.473	0.014*
		treatment		
	necropsy days	4	7.035	0.006*
		7		
		14		
	interaction test group–necropsy day		0.211	0.812
Re-epithelization rate	groups	control	23.026	0.014*
		treatment		
	necropsy days	4	8.028	0.000*
		7		
		14		
	interaction test group–necropsy day		0.393	0.008*

* statistically significant.

Table 3. Results of Tukey's post hoc HSD test with regard to differences in collagen density, angiogenesis, wound length, and the re-epithelialization rate between different necropsy days

Variable	Necropsy days	*p*-value
Collagen density	4–7	0.009*
	4–14	0.000*
	7–14	0.000*
Angiogenesis	4–7	0.007*
	4–14	0.994
	7–14	0.008*
Wound length	4–7	0.151
	4–14	0.000*
	7–14	0.000*
Re-epithelization rate	4–7	0.173
	4–14	0.004*
	7–14	0.176

* statistically significant.

Collagen density

The smallest average area of collagen density was in the control group on necropsy day 4 (0.04 ±0.03 mm^2), while the largest average area was in the control group on necropsy day 14 (0.29 ±0.33 mm^2). The two-way ANOVA revealed a statistically significant difference between all necropsy days. In addition, there was a statistically significant interaction between the study group and the necropsy day, influencing the average area of collagen density.

Angiogenesis

In terms of angiogenesis, it was found that the average number of blood vessels in the treatment group was lower than in the control group on all necropsy days. The peak in the control group was on necropsy day 7 (40.25 ±16.21). Furthermore, the multivariate analysis showed that there were significant differences in the average number of blood vessels between the treatment group and the control group, and between the necropsy days.

Wound length

The shortest average wound length was found in the control group on necropsy day 14 (2.18 ±4.23 mm), while the longest was found in the treatment group on necropsy day 4 (12.73 ±4.96 mm). The multivariate analysis revealed that there were significant differences in the average wound length between the treatment group and the control group, and between the necropsy days.

Re-epithelialization rate

The lowest average re-epithelialization rate was found in the control group on necropsy day 4 (0.38 ±0.49%), while the highest average re-epithelialization rate was found in the control group on necropsy day 14 (84.84 ±30.31%). The two-way ANOVA showed that there was a statistically significant interaction between the study group and the necropsy day, influencing the average value of the re-epithelialization rate.

Discussion

This study found that the collagen density area, angiogenesis (the number of blood vessels), wound length, and the level of re-epithelialization were significantly different on different necropsy days. The values for collagen density and the re-epithelialization rate increased with time, which was influenced by the increased fibroblast activity required for wound healing in the granulation phase. Meanwhile, the length of the wound tended to decrease, reflecting that the longer a wound undergoes the wound healing process, the shorter the wound length is because of the wound contraction process. The angiogenesis value was at its highest on necropsy day 7, indicating the commencement of angiogenesis in the initial proliferation phase and its continuous rise in the middle proliferation phase until it attained its peak and started to decrease in order to prevent excessive scarring.

A study conducted by Ismardianita et al. found that the oral administration of the *Hydnophytum formicarum* plant extract had an inversely proportional relationship with the number of macrophages in the inflammatory phase of wound healing after tooth extraction.[9] Macrophages play an important role in the secretion of cytokines to attract fibroblasts that become an essential component in the proliferation phase.[10] As already known, fibroblasts are responsible for important actions that support wound healing, such as the production of collagen, angiogenesis, wound contraction, and re-epithelialization. However, these activities can cause the formation of scar tissue if they occur excessively.[4] Previous studies determined that decreasing the number of macrophages in the inflammatory phase could be correlated with the suppressed fibroblast activities.[9,10]

A study conducted by Velanita et al. found that the administration of the *Hydnophytum formicarum* plant extract orally at a dose of 4.65 mg was able to significantly increase the production of collagen fibers in wound healing in the oral cavity after tooth extraction.[11] This is in contrast to the results of the present study, which indicated that collagen density in the control and treatment groups did not present significantly different values, although there was a statistically significant interaction between the use of the extract and the necropsy day, affecting collagen density. This interaction indicates that the *Hydnophytum formicarum* plant extract may change the value of collagen density when used for 14 days. Differences between the results of the present collagen density analysis and previous studies are probably related to the choice of tissue morphometric analysis. In this study, a quantitative method was employed, using the image processing software, whereas previous research used qualitative analyses, which tend to be more subjective.[12] Another potential cause of differences between the present study and previous research is the concentration of the administered extract; in the current study, pure extract without dilution was used. Moreover, the extract was applied topically, whereas in previous studies, it was administered orally.[9,11]

With regard to angiogenesis, this study indicates that the topically applied *Hydnophytum formicarum* plant extract can significantly reduce angiogenesis that occurs during the early, middle and late proliferation phases. This result is consistent with research by Ismardianita et al., who concluded that the oral administration of the *Hydnophytum formicarum* plant extract at a dose of 6.2 mg was able to significantly reduce angiogenesis in the proliferation phase.[9] By contrast, research conducted by Putri and Ismardianita found that the orally administered *Hydnophytum formicarum* plant extract at a dose of 4.65 mg was able to significantly increase angiogenesis.[13] Wound healing with minimal to no scarring occurs in the oral mucosa and the fetal skin in the uterus, presenting less angiogenesis as compared to wound healing in the skin; however, the capillaries become mature more rapidly. Many new capillaries in the healing wounds in the skin do not function effectively.[14] Histopathologically, less angiogenesis was observed in the treatment group in this study, but the blood vessels were more mature (Fig. 2).

Several clinical studies have concluded that excessive angiogenesis is associated with the formation of keloids. In addition, previous animal studies suggested that the partial inhibition of angiogenesis resulted in less hypertrophic scarring.[14] This effect occurs because of the presence of pericytes that stabilize the newly formed blood vessels. Therefore, the extent of angiogenesis parallels the number of pericytes. Pericytes play a role in myofibroblast transition, which affects the fibrosis process; thus, reducing angiogenesis leads to a decrease in the number of pericytes and suppresses the formation of excessive scar tissue.[14,15]

In the initial proliferation phase in this study, the re-epithelialization rate in the treatment group was higher than in the control group. However, in the middle and late proliferation phases, the re-epithelialization rate in the treatment group was lower than in the control group. Statistical analysis determined that the difference in the re-epithelialization rate between the control and treatment groups was significant, and that there was a significant interaction between the study group and the necropsy

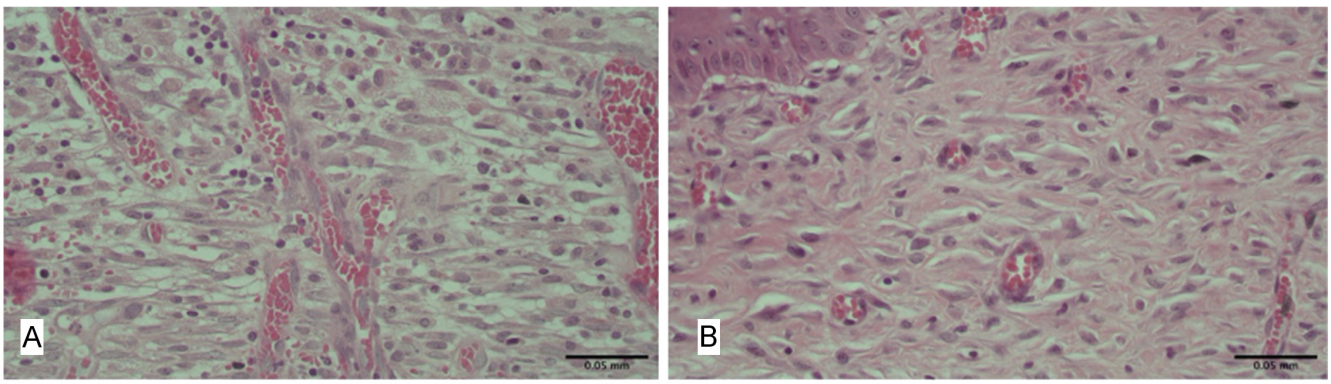

Fig. 2. Histopathological observation of angiogenesis on necropsy day 7
A – treated group; B – control group.

day, influencing the re-epithelialization rate. The re-epithelialization process is closely related to wound length. In this study, the wound length results were inversely proportional to the rate of re-epithelialization. Research on the effect of the *Hydnophytum formicarum* plant extract on re-epithelialization and wound length has not been conducted yet, but based on the prior knowledge of the wound healing process, it can be suggested that the re-epithelialization rate should be directly proportional to fibroblast activities. On the other hand, wound length is inversely proportional to the level of re-epithelialization and fibroblast activities.[10] This study concluded that the use of the *Hydnophytum formicarum* plant extract could significantly reduce the re-epithelialization rate if used for a sufficient period of time. Meanwhile, the observation of wound length indicated that the treatment group had significantly greater wound length values throughout the granulation phase. This led to the conclusion that the *Hydnophytum formicarum* plant extract has a significant effect on reducing wound length.

The production of collagen by fibroblasts in a balanced condition plays an important role in wound healing. Collagen deficiency can lead to chronic wounds, resulting in prolonged re-epithelialization, whereas excessive collagen production can lead to hypertrophic scarring.[4] The use of hypertrophic scar-preventing agents can have side effects if it is not properly timed. This initial study succeeded in confirming the ability of the *Hydnophytum formicarum* plant extract to suppress factors that cause scar tissue, but it was accompanied by a decrease in the re-epithelialization rate, which affected wound length. Therefore, further research is needed on this plant extract with different application times in the wound healing phase. One example of a wound care agent that is used topically to prevent scarring is silicone gel. The application of silicone gel is carried out when the wound has undergone full epithelialization, since it is considered that the material has an unfavorable effect if it is applied on an open wound.[16]

The systemic use of active ingredients administered via the oral route or with an intravenous injection has several drawbacks, such as toxicity on other organs, and requires higher doses to achieve a therapeutic effect. On the contrary, the advantage of the topical use of active ingredients consists in their continuous application while preventing them from being metabolized hepatically and in the gastrointestinal tract.[17] Based on these reasons, this study was conducted using active ingredients administered topically.

As reported in previous studies, the use of antibiotics and analgesics can influence the wound healing process. Antibiotics can suppress interleukin 1 beta (IL-1β), C-C motif chemokine ligand 2 (CCL2) and interferon alpha/beta (IFN-α/β), thereby leading to slower wound healing. Studies on the effect of non-steroidal anti-inflammatory drugs (NSAIDs) on wound healing provided conflicting results, yet the role of NSAIDs in inhibiting the COX pathway, which is related to the proliferation phase, is undeniable. Antibiotics and analgesics were not used in this study to prevent bias. In general, this study demonstrated normal morphology in the wound healing process in both groups. Also, there were no signs of secondary infection in any of the rats.

Conclusions

The topical use of the *Hydnophytum formicarum* plant extract affected the formation of scar tissue, as demonstrated by the positive area of collagen, the extent of angiogenesis, wound length, and the re-epithelialization rate in the early, middle and final granulation phases. The inhibition of angiogenesis through the application of *Hydnophytum formicarum* was probably related to the formation of scar tissue in the wound. However, the plant extract showed the ability to reduce the re-epithelialization rate, which affected wound length. Accordingly, further research is needed on this plant extract with different application times in the wound healing phase to achieve an optimal anti-scar tissue effect with minimal unwanted complications.

Ethics approval and consent to participate

The experiment was approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia (protocol No. 20-07-0783), as well as Dr. Cipto Mangunkusumo National Central Public Hospital, Jakarta, Indonesia.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Nissia Ananda https://orcid.org/0000-0003-2445-1618
Dwi Ariawan https://orcid.org/0000-0001-6407-4576
Vetnizah Juniantito https://orcid.org/0000-0002-0456-0844

References

1. Kibe T, Koga T, Nishihara K, et al. Examination of the early wound healing process under different wound dressing conditions. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2017;123(3):310–319. doi:10.1016/j.oooo.2016.10.023
2. Khezri K, Farahpour MR, Rad SM. Accelerated infected wound healing by topical application of encapsulated rosemary essential oil into nanostructured lipid carriers. *Artif Cells Nanomed Biotechnol.* 2019;47(1):980–988. doi:10.1080/21691401.2019.1582539
3. Wang P, Shu B, Xu Y, et al. Basic fibroblast growth factor reduces scar by inhibiting the differentiation of epidermal stem cells to myofibroblasts via the Notch1/Jagged1 pathway. *Stem Cell Res Ther.* 2017;8(1):114. doi:10.1186/s13287-017-0549-7

4. Chen D, Hao H, Fu X, Han W. Insight into reepithelialization : How do mesenchymal stem cells perform? *Stem Cells Int*. 2016;2016:6120173. doi:10.1155/2016/6120173
5. Marshall CD, Hu MS, Leavitt T, Barnes LA, Lorenz HP, Longaker MT. Cutaneous scarring : Basic science, current treatments, and future directions. *Adv Wound Care (New Rochelle)*. 2018;7(2):29–45. doi:10.1089/wound.2016.0696
6. Nilugal KC, Perumal K, Ugander RE, Chittor AI. Evaluation of wound healing activity of Piper betle leaves and stem extract in experimental Wistar rats. *Am J Pharm Tech Res*. 2014;4(3):443–452. https://www.researchgate.net/publication/272503669_Evaluation_of_Wound_Healing_Activity_of_Piper_Betle_Leaves_and_Stem_Extract_In_Experimental_Wistar_Rats. Accessed December 16, 2020.
7. Wanda IF. Mengenal *Hydnophytum formicarum* Jack; Sarang Semut Koleksi Kebun Raya Batam. *Warta Kebun Raya*. 2016;14(1):31–40. https://www.researchgate.net/profile/Irvan-Wanda/publication/318552391_MENGENAL_HYDNOPHYTUM_FORMICARUM_JACK_SARANG_SEMUT_KOLEKSI_KEBUN_RAYA_BATAM/links/597005940f7e9b4417366f94/MENGENAL-HYDNOPHYTUM-FORMICARUM-JACK-SARANG-SEMUT-KOLEKSI-KEBUN-RAYA-BATAM.pdf. Accessed November 25, 2020.
8. Sant'anna Addor FA. Antioxidants in dermatology. *An Bras Dermatol*. 2017;92(3):356–362. doi:10.1590/abd1806-4841.20175697
9. Ismardianita E, Ellanda F, Agus SA, Putri SA. The effect of ethanol extract of sarang semut plant (*Hypnophytum formicarum*) to granulation tissue for wound healing after teeth extraction experimental research on marmot (*Cavia cobaya*). *UIP Health Med*. 2017;1(1):72–76. doi:10.7454/uiphm.v1i0.28
10. Panayi AC, Reitblat C, Orgill DP. Wound healing and scarring. In: Ogawa R, ed. *Total Scar Management: From Lasers to Surgery for Scars, Keloids, and Scar Contractures*. Singapore: Springer; 2020:3–16.
11. Velanita S, Ismardianita E, Pascawinata A. The effect of ant-plant (*Hydnophytum formicarum*) ethanol extract on collagen fibers for wound healing after tooth extraction in the guinea pig (*Cavia cobaya*). *Padjadjaran J Dent*. 2019;31(3):189–195. doi:10.24198/pjd.vol31no3.19328
12. Meyerholz DK, Beck AP. Principles and approaches for reproducible scoring of tissue stains in research. *Lab Invest*. 2018;98(7):844–855. doi:10.1038/s41374-018-0057-0
13. Putri SA, Ismardianita E. The effect of ethanol extract of sarang semut plant (*Hypnophytum formicarum*) towards angiogenesis for wound healing after teeth extraction: Experimental research on marmot (*Cavia cobaya*). *Padjadjaran J Dent*. 2016;28(3):177–182. doi:10.24198/pjd.vol28no3.13675
14. DiPietro LA. Angiogenesis and wound repair: When enough is enough. *J Leukoc Biol*. 2016;100(5):979–984. doi:10.1189/jlb.4MR0316-102R
15. Stapor PC, Sweat RS, Dashti DC, Betancourt AM, Murfee WL. Pericyte dynamics during angiogenesis: New insights from new identities. *J Vasc Res*. 2014;51(3):163–174. doi:10.1159/000362276
16. Greenwood JE, Wagstaff MJ, Mackie IP, Mustoe TA. Silicone action in the open wound: A hypothesis. *J Burn Care Res*. 2012;33(1):e17–e20. doi:10.1097/BCR.0b013e31823e68cc
17. Whittam AJ, Maan ZN, Duscher D, et al. Challenges and opportunities in drug delivery for wound healing. *Adv Wound Care (New Rochelle)*. 2016;5(2):79–88. doi:10.1089/wound.2014.0600

Assessing the bone-healing potential of bone marrow mesenchymal stem cells in jawbone osteoporosis in albino rats

Tasneem Soliman[1,2,A–F], Zoba Ali[1,A–F], Mohamed Zayed[2,A,E,F], Dina Sabry[3,4,C,E,F], Nermeen AbuBakr[1,5,A–F]

[1] Department of Oral Biology, Faculty of Dentistry, Cairo University, Egypt
[2] Department of Oral Biology, Faculty of Dentistry, Misr International University, Cairo, Egypt
[3] Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Egypt
[4] Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Badr University in Cairo, Badr, Egypt
[5] Stem Cells and Tissue Engineering Unit, Faculty of Dentistry, Cairo University, Egypt

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):75–83

Address for correspondence
Nermeen AbuBakr
E-mail: nermeen.abubakr@dentistry.cu.edu.eg

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on February 10, 2021
Reviewed on June 15, 2021
Accepted on June 21, 2021

Published online on March 4, 2022

Abstract

Background. Osteoporosis is one of the most common yet difficult to treat diseases. It affects millions of people and costs the health care systems billions worldwide. All of the available kinds of pharmacological treatment have multiple side effects, which is why a need for safer treatment options has emerged.

Objectives. This study aimed to assess the bone-healing potential of bone marrow mesenchymal stem cells (BM-MSCs) in jawbone osteoporosis in Wistar albino rats.

Material and methods. Osteoporosis was induced with a daily intraperitoneal injection of 200 µg/100 g dexamethasone for 1 month. The rats were then randomly distributed into 2 groups: the osteoporotic group (left untreated); and the BM-MSCs group (received an intravenous injection of 50 million cultured BM-MSCs). Half of the rats from each group were sacrificed 2 weeks and the other half 6 weeks after the introduction of treatment. Bone regeneration was assessed by means of dual-energy X-ray absorptiometry (DEXA), real-time polymerase chain reaction (RT-PCR), as well as the histopathological and histomorphometric analyses.

Results. As for the 1st sacrifice time, there were no significant differences between the osteoporotic and BM-MSCs groups with regard to all parameters except for bone mineral density (BMD), which was significantly higher in the BM-MSCs group. Regarding the 2nd sacrifice time, the DEXA analysis showed a significant increase in BMD in the BM-MSCs group ($p < 0.001$). The RT-PCR analysis showed a significant decrease in *RANKL* gene expression ($p < 0.001$) and a significant increase in *OPG* gene expression ($p < 0.001$) in the BM-MSCs group. In addition, the histopathological examination of the BM-MSCs group showed pronounced healing progress in the jawbone microarchitecture. The histomorphometric analysis also revealed that the bone area percentage significantly increased in the BM-MSCs group ($p < 0.001$).

Conclusions. This study proved that BM-MSCs could be effective in the treatment of osteoporosis.

Keywords: mesenchymal stem cells, bone regeneration, osteoporosis, bone marrow

Cite as
Soliman T, Ali Z, Zayed M, Sabry D, AbuBakr N. Assessing the bone-healing potential of bone marrow mesenchymal stem cells in jawbone osteoporosis in albino rats. *Dent Med Probl.* 2022;59(1):75–83. doi:10.17219/dmp/139200

DOI
10.17219/dmp/139200

Introduction

Osteoporosis is a "progressive skeletal systemic disease characterized by reduced bone mass and microarchitectural deterioration, with a subsequent rise in bone fragility and fracture susceptibility".[1] It is the most common bone disease in humans, representing a major public health problem worldwide.[2]

Osteoporosis causes the loss of bone mineral density (BMD) throughout the body, including the maxillary and mandibular bones. This reduced jawbone density leads to the increased porosity and accelerated resorption of the alveolar bone. Since the alveolar processes provide the teeth with support, their reduced bone density can have negative consequences on tooth stability.[3]

Glucocorticoid-induced osteoporosis may happen in 30–50% of patients on glucocorticoid therapy. It is the main iatrogenic cause of secondary osteoporosis. Glucocorticoids increase the production of macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-beta ligand (RANKL), and decrease the production of osteoprotegerin (OPG), subsequently increasing the number and activity of osteoclasts.[4]

The pharmacological treatment of osteoporosis has been proven to have many side effects. Oral bisphosphonates, such as ibandronate, alendronate and risedronate, can cause upper gastrointestinal irritation and flu-like symptoms.[5,6] Intravenous amino-bisphosphonates, such as ibandronate and zoledronate, are potentially nephrotoxic, and can cause hypocalcemia and atrial fibrillation. They also pose an increased risk of heart failure, which is mostly due to the increased incidence of hyperlipidemia, hypertension and peripheral artery disease among these patients.[5–7] Raloxifene can cause venous thromboembolism, whereas teriparatide has triggered concerns about osteosarcoma in animal studies.[5]

Mesenchymal stem cells (MSCs) obtained from bone marrow (BM) can be separated easily, have the ability to self-renew, proliferate and differentiate into multilineages, retain the 'homing' feature, and are characterized by long storage without major loss of potency, thus making them the first option in repairing bone and treating related diseases.[8,9]

Bone regeneration is a complex process involving the interrelation between adipogenic and osteogenic progenitor cells, which are both derived from BM.[10] It has been confirmed that BM-MSCs can be differentiated into osteoblasts and secrete many osteogenic factors after being cultured in vitro. They have also been proven to have great potential in bone and soft tissue repair in vivo. Moreover, they can migrate to the site of injury, creating an appropriate microenvironment for tissue repair.[11,12]

In this context, this study aimed to assess the bone healing potential of BM-MSCs in glucocorticoid-induced jawbone osteoporosis by evaluating BMD as a primary outcome in addition to the gene expression of *RANKL* and *OPG*, histopathological alterations, and the histomorphometric analysis of the jawbones as secondary outcomes.

Material and methods

This experiment was conducted at the animal house at the Faculty of Medicine of Cairo University, Egypt, after obtaining the approval of the Institutional Animal Care and Use Committee (IACUC) (CU-III-F-73-17).

Isolation and culture of BM-MSCs

Bone marrow was flushed out of the tibias of male albino rats with an approximate weight of 100–120 g and age of 6 weeks by using phosphate buffered saline (PBS) (Invitrogen, Grand Island, USA) and centrifuged at 1,000 rpm for 5 min. A total of 35 mL of the flushed BM cells was layered over 15 mL Ficoll-Paque™ (Invitrogen) and centrifuged at 400 × g for 35 min. The upper layer was discarded, leaving a mononuclear cell (MNC) layer at the interphase. This MNC layer was collected, washed twice in PBS, and centrifuged at 200 × g for 10 min at 10°C. The isolated BM-MSCs were cultured and propagated in 25-milliliter culture flasks with RPMI-1640 (Merck, Darmstadt, Germany) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Waltham, USA), 0.5% penicillin (Thermo Fisher Scientific) and streptomycin (Thermo Fisher Scientific). It was subsequently incubated at 5% CO_2 and 37°C until reaching 80–90% confluence within 14 days of culture.[13]

Identification of BM-MSCs in the culture

Bone marrow MSCs were characterized in accordance with the International Society for Cellular Therapy guidelines[14] in terms of their morphology, adherence, fluorescence-activated cell sorting (FACS) by assessing positivity for $CD90^+$, $CD105^+$ and $CD73^+$, and negativity for $CD14^-$, $CD34^-$ and $CD45^-$, and their capability to differentiate into osteoblasts, adipocytes and chondroblasts. The differentiation of BM-MSCs into ostoblasts was performed using a StemPro™ osteogenesis differentiation kit (Life Technologies, Carlsbad, USA); the cells were stained with the Alizarin Red S stain (Sigma-Aldrich, St. Louis, USA). Adipocyte differentiation was achieved with a StemPro™ adipogenesis differentiation kit (Life Technologies), and the cells were subsequently stained with the Oil Red O stain (Sigma-Aldrich). Chondroblast differentiation was performed using a StemPro™ chondrogenesis differentiation kit (Life Technologies), after which the cells were stained with the Alcian Blue stain (Sigma-Aldrich).

Sample size

The sample size was calculated using the G*power software (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). In terms of the primary outcome (BMD) based on Uejima et al., the results revealed 124.32 ±4.88 mg/cm^2 for the control bones and 139.35 ±4.63 mg/cm^2 for the BM-MSCs-injected

bones[15]; it was found that 3 rats per group with a total sample size of 12 rats was the appropriate sample size for the study (2 groups per each duration of 2 and 6 weeks). The power was 80% and the α error probability 0.05. The number was increased to 4 rats in each group to compensate for possible losses during the experiment for a total of 16 rats (4 × 4). The magnitude of the effect to be detected was estimated as mean and standard deviation (*M* ±*SD*) for the variable of interest, and was obtained from the scientific literature (Uejima et al.[15]).

Experimental animals

A total of 16 healthy male Wistar albino rats (*Rattus norvegicus*) with an approximate weight of 150–200 g and age of 3–4 months were obtained from the animal house at the Faculty of Medicine of Cairo University. The animals were housed in a controlled, sterile environment (temperature 23 ±5°C and 12-hour dark/light cycles). They had free access to a standard pellet diet and tap water ad libitum. They were maintained individually in stainless steel cages and kept under good ventilation. Each individual rat was considered an experimental unit within this study.

Study design

The animals were randomly divided using the Random Sequence Generator program (https://www.random.org/). Each animal was assigned a temporary random number. The rats were divided into 2 groups of 8 rats each (4 for each sacrifice date) as follows:

– the osteoporotic group consisted of 8 glucocorticoid-induced osteoporotic rats; these rats received no treatment and served as a positive control group;
– the BM-MSCs group consisted of 8 glucocorticoid-induced osteoporotic rats; these rats received a single intravenous injection of 50 million cultured BM-MSCs in PBS through the tail vein.[16]

Experimental procedure

The experiment was held at the animal house at the Faculty of Medicine of Cairo University. Four investigators were involved for each animal; the 1st investigator was the only one aware of group allocation. During the experimental procedure, the 2nd investigator was the only person to assess dosage administration for all animals. The 3rd investigator assessed the outcomes in a masked fashion, without knowing the groups. The statistician performed data analysis and was unaware of group allocation. All rats were intraperitoneally injected with 200 μg/100 g dexamethasone (Amriya Pharmaceuticals, Alexandria, Egypt) once daily for 30 days to induce osteoporosis.[17] Bone mineral density was measured at the jawbone to confirm that the model was successfully established. Both the rats in the BM-MSCs group and the rats in the untreated osteoporotic group were sacrificed 2 and 6 weeks after the introduction of treatment. The animals were sacrificed using an intraperitoneal overdose injection of an anesthetic mixture (ketamine/xylazine) (Sigma-Aldrich).[18] Each lower jaw was dissected and processed for the assessment of the experimental outcomes.

Experimental outcomes

Dual-energy X-ray absorptiometry (DEXA) (primary outcome)

The BMD of the mandibles was measured using dual-energy X-ray absorptiometry (DEXA). A Norland XR-46 DXA scanner (Norland Corp., Fort Atkinson, USA), equipped with the appropriate software for bone assessment, was utilized. The scan resolution was 0.5 × 0.5 mm and the scan speed was 60 mm/s. The analysis was carried out based on the image of the animal's jawbone on the screen, using a region of interest (ROI), which was defined to include the bone area below the first molar. The results were displayed automatically in g/cm^2.[19]

Real-time polymerase chain reaction (RT-PCR) (secondary outcome)

Total RNA was extracted for further real-time polymerase chain reaction (RT-PCR) analysis. RNA was isolated with an RNeasy Micro Kit (Cat. No./ID: 74004; Qiagen, Hilden, Germany). A total of 20 ng of the isolated total RNA was used to develop cDNA by means of a high-capacity cDNA Reverse Transcription Kit (Cat. No: 4368814; Applied Biosystems, Foster City, USA). The PCR analysis was performed using a SYBR® Green Master Mix kit (Cat. No: 4344463; Applied Biosystems) with the StepOnePlus™ Real-Time PCR System (Applied Biosystems), according to standard protocols. Briefly, the RT-PCR thermal profile was programmed as follows: 10 min at 45°C for reverse transcription; 2 min at 98°C for the inactivation of reverse transcriptase (RT); and initial denaturation with 40 cycles of 10 s at 98°C, 10 s at 55°C and 30 s at 72°C for the amplification step. The relative quantity (RQ) values for each target gene were measured according to the calculation of ΔΔCt. The calculation of the RQ values for the studied genes was performed by $2^{-\Delta\Delta Ct}$ normalization to the housekeeping gene *GADPH*. The primers for *RANKL*, *OPG* and *GADPH* are shown in Table 1.

Table 1. Primer sequences specific for each gene

Gene symbol	Primer sequences from 5' to 3'
RANKL	F: ACC AGC ATC AAA ATC CCA AG R: TTT GAA AGC CCC AAA GTA CG
OPG	F: GTT CTT GCA CAG CTT CAC CA R: AAA CAG CCC AGT GAC CAT TC
GAPDH	F: GACGGCCGCATCTTCTTGA R: CACACCGACCTTCACCATTTT

Histopathological examination (secondary outcome)

The specimens were decalcified for 4 weeks, dehydrated in ascending grades of alcohol, cleared in xylol, and embedded in paraffin blocks. Serial sections of a thickness of 5–6 μm were cut, mounted on glass slides, and stained with hematoxylin and eosin (H&E) for a routine histopathological examination.

Histomorphometric analysis (secondary outcome)

The area percentage of bone in the region below the first molar for each specimen was measured. The data was obtained using a Leica Qwin 500 image analyzer computer system (Wetzlar, Germany). The area and area percentage of bone trabeculae were measured with an objective lens magnification of ×20 (a total magnification of ×200). Five fields were measured for each specimen. The bone area percentage was calculated in relation to a standard measuring frame having an area of 118,476.6 μm^2.

Statistical analysis

The data was coded and processed using the IBM SPSS Statistics for Windows software, v. 24.0 (IBM Corp., Armonk, USA). The data was presented as $M \pm SD$. The Kolmogorov–Smirnov test showed that the data was normally distributed; thus, comparisons between the 2 groups were performed using the independent t test. A p-value ≤0.05 was considered statistically significant.

Results

Morphological characterization of BM-MSCs

The morphology of BM-MSCs was observed under an inverted microscope (Invitrogen, Waltham, USA). Bone marrow MSCs were expanded successfully and adhered to culture flasks, showing a heterogenous population, displaying fibroblast-like morphology and forming a confluent monolayer at 14 days of culture (Fig. 1(I)A). Bone marrow MSCs were differentiated into osteoblasts, stained with Alizarin Red S (Fig. 1(I)B), adipocytes, stained with Oil Red O (Fig. 1(I)C), and chondroblasts, stained with Alcian Blue (Fig. 1(I)D).

Phenotypic characterization of BM-MSCs

The phenotypic FACS analysis revealed that BM-MSCs were positive for CD90$^+$ (95.2%), CD105$^+$ (94.0%) and CD73$^+$ (96.4%), and negative for CD14$^-$ (0.6%), CD34$^-$ (1.6%) and CD45$^-$ (1.4%) (Fig. 1(II)).

DEXA results

A significant increase in BMD was observed in the BM-MSCs group as compared to the osteoporotic group at the 1st and 2nd sacrifice times ($p < 0.001$) (Table 2).

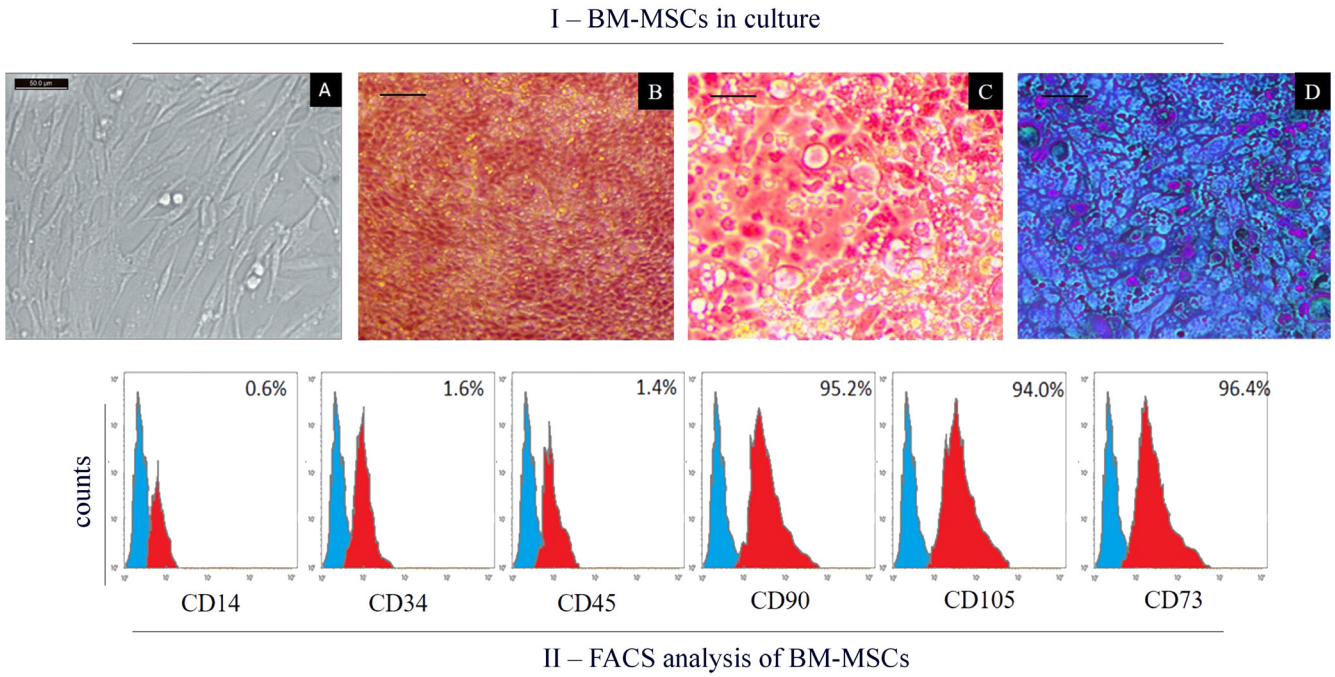

Fig. 1. (I) Bone marrow mesenchymal stem cells (BM-MSCs) in culture: A – plastic adherence and spindle-shaped morphology at 14 days of culture (scale bar: 50 μm); B – BM-MSCs differentiated into osteoblasts, stained with Alizarin Red S (scale bar: 100 μm); C – BM-MSCs differentiated into adipocytes, stained with Oil Red O (scale bar: 100 μm); D – BM-MSCs differentiated into chondroblasts, stained with Alcian Blue (scale bar: 100 μm); (II) fluorescence-activated cell sorting (FACS) analysis of BM-MSCs: positive for CD90$^+$ (95.2%), CD105$^+$ (94.0%) and CD73$^+$ (96.4%), and negative for CD14$^-$ (0.6%), CD34$^-$ (1.6%) and CD45$^-$ (1.4%)

Table 2. Dual-energy X-ray absorptiometry (DEXA), real-time polymerase chain reaction (RT-PCR) and histomorphometric results

Analysis	1st sacrifice time			2nd sacrifice time		
	osteoporotic group	BM-MSCs group	*p*-value	osteoporotic group	BM-MSCs group	*p*-value
DEXA (BMD [g/cm^2])	0.05 ±0.01	0.09 ±0.01	<0.001*	0.02 ±0.01	0.10 ±0.02	<0.001*
RANKL gene expression [AU]	1.64 ±0.52	1.34 ±0.47	>0.05	3.38 ±0.42	0.26 ±0.15	<0.001*
OPG gene expression [AU]	0.60 ±0.15	0.66 ±0.19	>0.05	0.14 ±0.02	1.59 ±0.21	<0.001*
RANKL/OPG ratio	2.80 ±0.94	2.20 ±1.02	>0.05	24.70 ±6.80	0.17 ±0.04	<0.001*
Histomorphometry (bone area percentage [%])	39.99 ±4.90	41.07 ±7.70	>0.05	22.70 ±2.10	85.59 ±2.60	<0.001*

Data expressed as mean ± standard deviation (*M* ±*SD*) for 4 rats ($n = 4$). BM-MSCs – bone marrow mesenchymal stem cells; BMD – bone mineral density; AU – arbitrary unit (relative expression); * statistically significant.

RT-PCR results

At the 1st sacrifice time there was no significant difference between the osteoporotic and BM-MSCs groups in terms of gene expression ($p > 0.05$). However, at the 2nd sacrifice time there was a significant decrease in *RANKL* and a significant increase in *OPG* gene expression in the BM-MSCs group as compared to the osteoporotic group ($p < 0.001$) (Table 2).

RANKL/OPG ratio in the study groups

At the 1st sacrifice time there was no significant difference between the osteoporotic and BM-MSCs groups in terms of *RANKL/OPG* ratio ($p > 0.05$). However, at the 2nd sacrifice time there was a significant decrease in the *RANKL/OPG* ratio in the BM-MSCs group as compared to the osteoporotic group ($p < 0.001$) (Table 2).

Histopathological results

The histopathological examination of the alveolar bone in the osteoporotic group at the 1st sacrifice time showed clear signs of osteoporosis, such as multiple osteoporotic cavities, scalloped resorptive pits and widened BM cavities surrounded by thin bone trabeculae. Bone marrow cavities showed areas of extravasated red blood cells (RBCs). Cellular degeneration was demonstrated by the diminished osteoblastic lining of BM cavities, the presence of shrunken osteocytes, empty lacunae, and bone areas free of osteocytes. Multinucleated osteoclasts in Howship's lacunae and reversal lines were also observed (Fig. 2).

The histopathological examination of the alveolar bone in the BM-MSCs group at the 1st sacrifice time revealed some signs of healing, even though BM cavities were still wide. Bone lamellae showed a more homogenous and better organized architecture. Chronic inflammatory cell infiltration was detected. In addition, congested and dilated blood vessels were present. Some of widened BM cavities regained their osteoblastic lining, although they were still surrounded by thin bone trabeculae. A few osteoporotic and resorptive cavities were still observed. Areas of fatty degeneration were spotted where BM cavities contained multiple adipocytic cells. Regions of newly formed woven bone were noticed with disorganized fibers and osteocytes that were rounder, larger and less regularly spaced than the ones in the lamellar bone. Areas of parallel fibered bone were also found. It appeared as a transitional

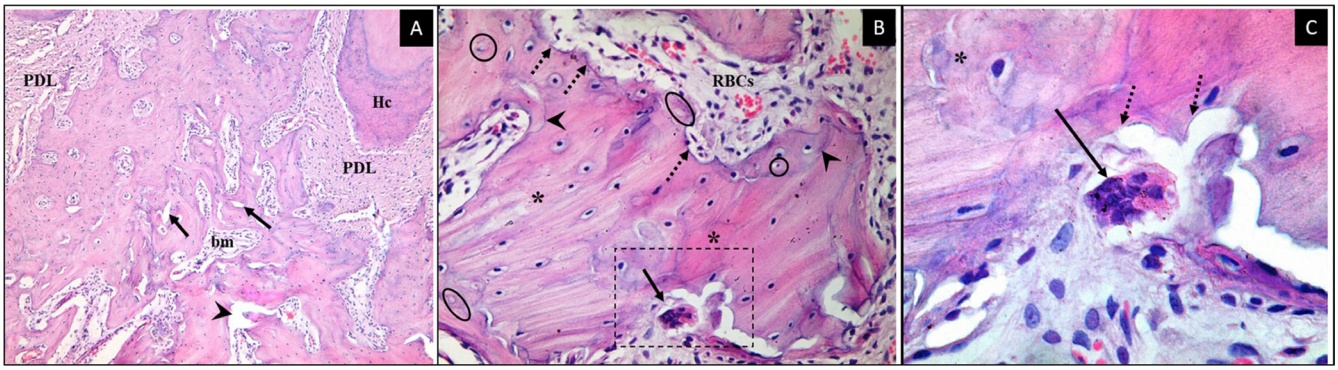

Fig. 2. Photomicrographs of the osteoporotic group at the 1st sacrifice time

A – multiple bone marrow (BM) cavities (bm), some with diminished osteoblastic lining (arrow head), multiple osteoporotic cavities (arrows), hypercementosis (Hc), and the periodontal ligaments (PDL) with degenerated areas (×100 magnification); B – widening and scalloping of BM cavities (dotted arrows) with extravasated red blood cells (RBCs), osteocytes shrunken in their lacunae (circles), empty lacunae (ovals), areas free of osteocytes (asterisks), reversal lines (arrow heads), and a multinucleated osteoclast in a Howship's lacuna (arrow) (×400 magnification); C – higher magnification of the previous figure, showing a multinucleated osteoclast (arrow) in a Howship's lacuna (dotted arrows) (×1,000 magnification).

bone tissue between the newly formed woven bone and the lamellar bone (Fig. 3).

The histopathological examination of the alveolar bone in the osteoporotic group at the 2nd sacrifice time presented augmented signs of osteoporosis. Marrow spaces exhibited diminished osteoblastic lining. Wide BM cavities revealed some fatty degeneration with the presence of multiple chronic inflammatory cells. Multiple osteoporotic cavities and malorganized trabecular lamellae were detected. Osteocytes shrunken in their lacunae and some other empty lacunae were observed. Bone resting lines were also noticed (Fig. 4).

The histopathological examination of the alveolar bone in the BM-MSCs group at the 2nd sacrifice time displayed a great extent of bone healing. Bone marrow cavities returned to their normal size and regained their osteoblastic lining. Thick trabecular bone areas containing normal healthy osteocytes of normal size and shape, and resting in their lacunae were noticed. Bone resting lines surrounding marrow spaces were observed. Vascular spaces were noticed near the periodontal ligaments (PDLs). Some vascular spaces turned into secondary osteons surrounded by reversal lines. In addition, scalloped bone reversal lines were noticed, demonstrating the active bone formation and healing process (Fig. 5).

Histomorphometric results

The comparison of the osteoporotic and BM-MSCs groups revealed that the bone area percentage values were not statistically significantly different at the 1st sacrifice time ($p > 0.05$), but were statistically significantly different at the 2nd sacrifice time ($p < 0.001$) (Table 2).

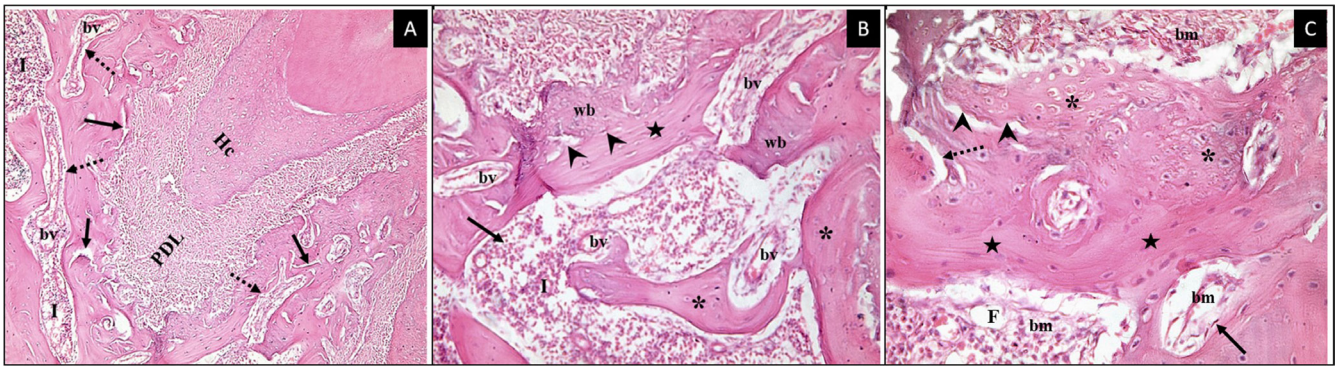

Fig. 3. Photomicrographs of the BM-MSCs group at the 1st sacrifice time

A – multiple wide BM cavities (dotted arrows) with some dilated blood vessels (bv) and chronic inflammatory cells (I), some osteoporotic cavities (arrows), hypercementosis (Hc), and a normal periodontal ligament (PDL) (×100 magnification); B – a large BM cavity (arrow) containing dilated blood vessels (bv) and chronic inflammatory cells (I), surrounded by islands of thin bone trabeculae (asterisks), with a line demarcating (arrow heads) areas of newly formed woven bone (wb) and an area of parallel fibered bone (star) (×200 magnification); C – widened BM cavities (bm) with osteoblastic lining (arrow), few areas of fatty degeneration (F) and a resorptive bony cavity (dotted arrow), areas of newly formed woven bone (asterisks) with large osteocytes, demarcated by a scalloped line (arrow heads), and areas of parallel fibered bone (stars) (×400 magnification).

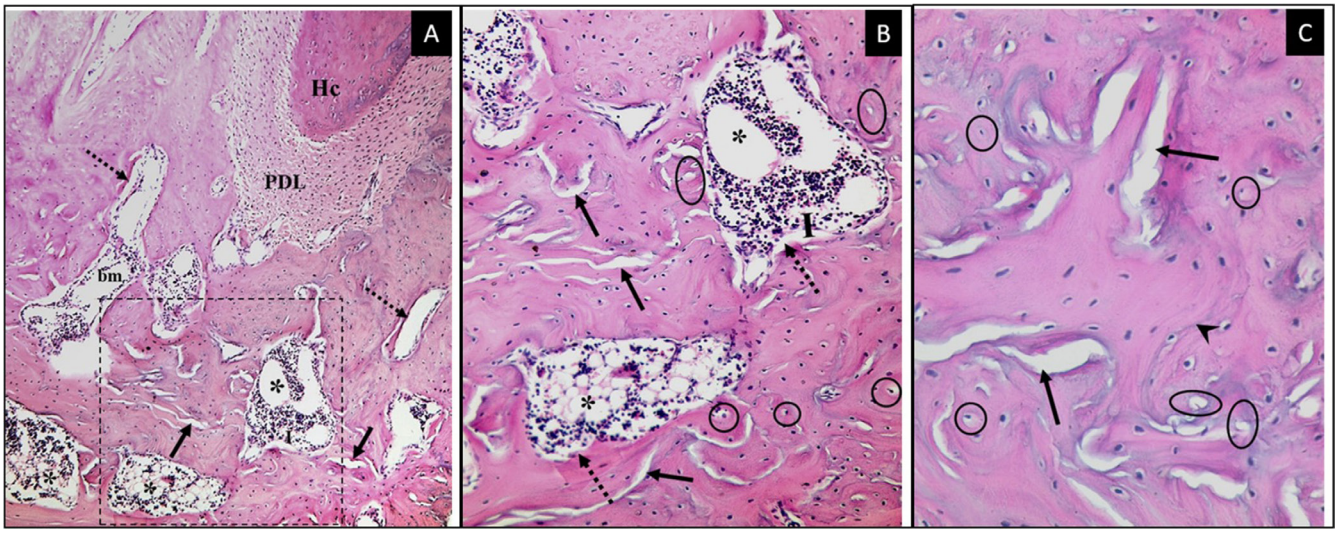

Fig. 4. Photomicrographs of the osteoporotic group at the 2nd sacrifice time

A – wide marrow spaces (bm) with diminished osteoblastic lining (dotted arrows), fatty degeneration (asterisks), chronic inflammatory cells (I), osteoporotic cavities (arrows), hypercementosis (Hc), and a normal periodontal ligament (PDL) (×100 magnification); B – higher magnification of the previous figure, showing wide marrow spaces (dotted arrows), fatty degeneration (asterisks), chronic inflammatory cells (I), multiple osteoporotic cavities of varying sizes (arrows), osteocytes shrunken in their lacunae (circles), and empty lacunae (ovals) (×200 magnification); C – multiple wide osteoporotic cavities (arrows), shrunken osteocytes (circles), empty lacunae (ovals), and a bone resting line (arrow head) (×400 magnification).

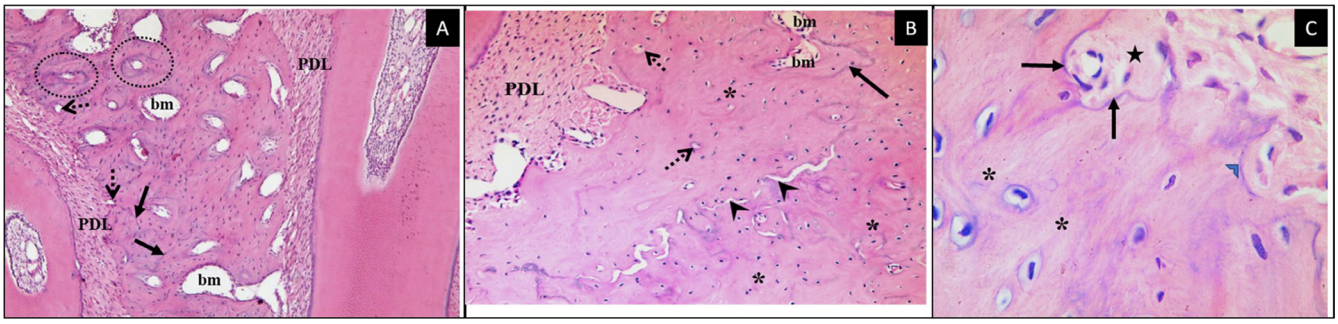

Fig. 5. Photomicrographs of the BM-MSCs group at the 2nd sacrifice time

A – alveolar marrow spaces with a complete osteoblastic lining (bm), resting lines (arrows), vascular spaces (dotted arrows), secondary osteons (dotted circles), and normal periodontal ligaments (PDL) (×100 magnification); B – BM cavities with an osteoblastic lining (bm), osteocytes of normal size and shape (asterisks), vascular channels (dotted arrows), a resting line (arrow), reversal lines (arrow heads), and a normal periodontal ligament (PDL) (×200 magnification); C – normal osteocytes in their lacunae (asterisks) and bone reversal lines (arrows) denoting newly formed bone matrix (star) (×1,000 magnification).

Discussion

The seriousness of osteoporosis lies in it being a totally asymptomatic illness that often remains undiagnosed until it is manifested as a fracture.[2] All of the available kinds of pharmacological treatment are accompanied by multiple side effects, which is why a need for safer treatment options has emerged.[20] Hence, this study aimed to assess the bone healing potential of BM-MSCs in glucocorticoid-induced jawbone osteoporosis.

In the current work, osteoporosis was examined in the mandibular jawbones of adult rats. This is in accordance with Jonasson and Rythén, who stated that the rate of bone turnover in the mandibular alveolar processes might be the fastest in the body; therefore, the initial signs of osteoporosis could be revealed there the earliest.[21]

During the study period, BMD significantly deteriorated in the osteoporotic group. This finding is consistent with Cao et al., who reported that BMD significantly decreased in osteoporotic rats.[22]

At the 2nd sacrifice time, *RANKL* gene expression was significantly elevated in the osteoporotic group. This is in agreement with Eghbali-Fatourechi et al., who proved a significant upregulation of *RANKL* in osteoporotic postmenopausal women.[23] On the other hand, *OPG* gene expression was significantly decreased. This is in accordance with a clinical study in which the delayed phase of osteoporosis caused *OPG* levels to decrease.[24]

In the current investigation, the histopathological changes in the osteoporotic group became more noticeable at the 2nd sacrifice time. These observations resemble the findings of previous authors, who observed marked histopathological alterations in the mandibular alveolar bone spongiosa of osteoporotic rats.[17,25] Moreover, the great increase in BM fat content observed at the 2nd sacrifice time was demonstrated by former studies, which reported that an increase in circulating glucocorticoids caused fatty tissue infiltration and the expansion of BM adipose tissue.[26,27]

In this study, the histomorphometric analysis confirmed the histopathological architectural changes in the osteoporotic group. This coincides with the findings of Abuohashish et al., who determined that the morphometric parameters of femur bones were significantly decreased in osteoporotic rats.[28]

In the present work, after injecting the osteoporotic rats with BM-MSCs, BMD significantly increased along the 2 sacrifice periods. This is quite similar to the observations of Hsiao et al., who confirmed that BM-MSCs migrated to bone after an intravenous injection, leading to an increased BMD and restored bone volume in an ovariectomized mouse model.[29] This might be attributed to the ability of the transplanted MSCs to migrate to the site of injury and produce immunomodulatory cytokines and growth factors, helping local cells to recover and inducing the recruitment of new cells in the area.[8]

In the BM-MSCs group, *RANKL* gene expression significantly decreased at the 2nd sacrifice time as compared to the osteoporotic group. These results are supported by Li et al., who found BM-MSCs to alleviate the symptoms of arthritis mainly by decreasing the levels of *RANKL* gene expression.[30] On the other hand, the level of *OPG* gene expression showed a significant increase. This coincides with the results of Oshita et al., who reported that human MSCs produced OPG continuously, at both the mRNA and protein levels, which inhibited the osteoclastogenesis process.[31]

In the current work, the histopathological findings in the BM-MSCs group revealed gradual improvement between the 1st and 2nd sacrifice times. This is in agreement with Freitas et al., who demonstrated that the newly formed bone was observed 4 weeks after the injection of BM-MSCs in rat calvarial defects.[32] This is also supported by Kim et al., who proved that MSCs reduced the progression of senile osteoporosis by sustaining osteocalcin levels in the circulation, which resulted in improved bone microarchitecture.[33]

Concerning the histomorphometric analysis, the BM-MSCs group showed a significant progressive increase in bone volume at the 2nd sacrifice time. This is in agreement with de Melo Ocarino et al., who found that the greatest bone volume was achieved 2 months after injecting the osteoporotic rats with differentiated BM-MSCs.[34]

The osteoporosis model should be further studied on animals with bone morphology closer to humans, as osteogenic healing in rats far exceeds that of a human. Also, more research techniques are needed to fully understand the healing mechanism of BM-MSCs in the treatment of osteoporosis. Furthermore, more clinical trials should be conducted to determine the proper effective human dosage of BM-MSCs in treating osteoporosis.

Conclusions

After assessing all the DEXA, RT-PCR, histopathological, and histomorphometric results, it was confirmed that BM-MSCs had a positive effect on bone healing potential. It was also shown that the healing progress was achieved gradually along the experiment duration. Therefore, it can be concluded that BM-MSCs could act as an effective treatment option for osteoporosis. However, further experiments with larger sample sizes are recommended to confirm these results.

Ethics approval and consent to participate

This experiment was conducted at the animal house at the Faculty of Medicine of Cairo University, Egypt, after obtaining the approval of the Institutional Animal Care and Use Committee (IACUC) (CU-III-F-73-17).

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Tasneem Soliman https://orcid.org/0000-0003-0908-2113
Zoba Ali https://orcid.org/0000-0003-1713-8586
Mohamed Zayed https://orcid.org/0000-0001-8453-6088
Dina Sabry https://orcid.org/0000-0002-6720-3385
Nermeen AbuBakr https://orcid.org/0000-0003-2962-0070

References

1. Kanis JA, Melton LJ 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. *J Bone Miner Res*. 1994;9(8):1137–1141. doi:10. 1002/jbmr.5650090802
2. Compston J, Cooper A, Cooper C, et al. UK clinical guideline for the prevention and treatment of osteoporosis. *Arch Osteoporos*. 2017;12(1):43. doi:10.1007/s11657-017-0324-5
3. Anil S, Preethanath RS, AlMoharib HS, Kamath KP, Anand PS. Impact of osteoporosis and its treatment on oral health. *Am J Med Sci*. 2013;346(5):396–401. doi:10.1097/MAJ.0b013e31828983da
4. Swanson C, Lorentzon M, Conaway HH, Lerner UH. Glucocorticoid regulation of osteoclast differentiation and expression of receptor activator of nuclear factor-kappaB (NF-kappaB) ligand, osteoprotegerin, and receptor activator of NF-kappaB in mouse calvarial bones. *Endocrinology*. 2006;147(7):3613–3622. doi:10.1210/en.2005-0717
5. Reid IR. Efficacy, effectiveness and side effects of medications used to prevent fractures. *J Intern Med*. 2015;277(6):690–706. doi:10.1111/joim.12339
6. Tu KN, Lie JD, Wan CKV, et al. Osteoporosis: A review of treatment options. *P T*. 2018;43(2):92–104. PMID:29386866. PMCID:PMC5768298.
7. Rubin KH, Möller S, Choudhury A, et al. Cardiovascular and skeletal safety of zoledronic acid in osteoporosis observational, matched cohort study using Danish and Swedish health registries. *Bone*. 2020;134:115296. doi:10.1016/j.bone.2020.115296
8. Wang C, Meng H, Wang X, Zhao C, Peng J, Wang Y. Differentiation of bone marrow mesenchymal stem cells in osteoblasts and adipocytes and its role in treatment of osteoporosis. *Med Sci Monit*. 2016;22:226–233. doi:10.12659/msm.897044
9. Macías I, Alcorta-Sevillano N, Rodríguez CI, Infante A. Osteoporosis and the potential of cell-based therapeutic strategies. *Int J Mol Sci*. 2020;21(5):1653. doi:10.3390/ijms21051653
10. Liu HY, Wu ATH, Tsai CY, et al. The balance between adipogenesis and osteogenesis in bone regeneration by platelet-rich plasma for age-related osteoporosis. *Biomaterials*. 2011;32(28):6773–6780. doi:10.1016/j.biomaterials.2011.05.080
11. Bronckers AL, Sasaguri K, Engelse MA. Transcription and immunolocalization of Runx2/Cbfa1/Pebp2alphaA in developing rodent and human craniofacial tissues: Further evidence suggesting osteoclasts phagocytose osteocytes. *Microsc Res Tech*. 2003;61(6):540–548. doi:10.1002/jemt.10377
12. Jiang Y, Zhang P, Zhang X, Lv L, Zhou Y. Advances in mesenchymal stem cell transplantation for the treatment of osteoporosis. *Cell Prolif*. 2021;54(1):e12956. doi:10.1111/cpr.12956
13. AbuBakr N, Haggag T, Sabry D, Salem ZA. Functional and histological evaluation of bone marrow stem cell-derived exosomes therapy on the submandibular salivary gland of diabetic Albino rats through TGFβ/Smad3 signaling pathway. *Heliyon*. 2020;6(4):e03789. doi:10.1016/j.heliyon.2020.e03789
14. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8(4):315–317. doi:10.1080/14653240600855905
15. Uejima S, Okada K, Kagami H, Taguchi A, Ueda M. Bone marrow stromal cell therapy improves femoral bone mineral density and mechanical strength in ovariectomized rats. *Cytotherapy*. 2008;10(5):479–489. doi:10.1080/14653240802071616
16. Chutkerashvili G, Menabde G, Chutkerashvili K, Dotiashvili D, Amiranashvili I. The treatment of osteoporosis through transplantation of bone marrow stem cells in the experiments performed on rats. *Georgian Med News*. 2012;3(204):88–92. PMID:22573756.
17. Ezzat BA, Abbass MM. The ability of H1 or H2 receptor antagonists or their combination in counteracting the glucocorticoid-induced alveolar bone loss in rats. *J Oral Pathol Med*. 2014;43(2):148–156. doi:10.1111/jop.12104
18. Leary S, Underwood W, Anthony R, et al.; the Panel of Euthanasia. *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition*. Schaumburg, IL: American Veterinary Medical Association; 2013.
19. Gao SG, Li KH, Xu M, et al. Bone turnover in passive smoking female rat: Relationships to change in bone mineral density. *BMC Musculoskelet Disord*. 2011;12:131. doi:10.1186/1471-2474-12-131
20. Lin H, Sohn J, Shen H, Langhans MT, Tuan RS. Bone marrow mesenchymal stem cells: Aging and tissue engineering applications to enhance bone healing. *Biomaterials*. 2019;203:96–110. doi:10.1016/j.biomaterials.2018.06.026
21. Jonasson G, Rythén M. Alveolar bone loss in osteoporosis: A loaded and cellular affair? *Clin Cosmet Investig Dent*. 2016;8:95–103. doi:10.2147/CCIDE.S92774
22. Cao H, Zhang Y, Qian W, et al. Effect of icariin on fracture healing in an ovariectomized rat model of osteoporosis. *Exp Ther Med*. 2017;13(5):2399–2404. doi:10.3892/etm.2017.4233
23. Eghbali-Fatourechi G, Khosla S, Sanyal A, Boyle WJ, Lacey DL, Riggs BL. Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. *J Clin Invest*. 2003;111(8):1221–1230. doi:10.1172/JCI17215
24. Gurban C, Zosin I, Sfrijan F, et al. The OPG/sRANKL system and the low bone mineral density in postmenopausal osteoporosis. *Acta Endo (Buc)*. 2009;5(1):27–40. doi:10.4183/aeb.2009.27

25. Abu Taleb OM, Wissa MY, Abou El Nour RK, Awad HA, Moussa NM. Potential effectiveness of exenatide in experimentally-induced osteoporosis. *Egypt Rheumatol*. 2020;42(1):57–62. doi:10.1016/j.ejr.2019.06.003
26. Cawthorn WP, Scheller EL, Parlee SD, et al. Expansion of bone marrow adipose tissue during caloric restriction is associated with increased circulating glucocorticoids and not with hypoleptinemia. *Endocrinology*. 2016;157(2):508–521. doi:10.1210/en.2015-1477
27. Sherif H, El Masry NA, Kawana KY, Khalil NM. Effect of bisphosphonates on the alveolar bone of rats with glucocorticoids induced osteoporosis. *Alexandria Dent J*. 2019;44(3):65–70. doi:10.21608/ADJALEXU.2019.63560
28. Abuohashish HM, Ahmed MM, Al-Rejaie SS, Eltahir KE. The antidepressant bupropion exerts alleviating properties in an ovariectomized osteoporotic rat model. *Acta Pharmacol Sin*. 2015;36(2):209–220. doi:10.1038/aps.2014.111
29. Hsiao FSH, Cheng CC, Peng SY, et al. Isolation of therapeutically functional mouse bone marrow mesenchymal stem cells within 3 h by an effective single-step plastic-adherent method. *Cell Prolif*. 2010;43(3):235–248. doi:10.1111/j.1365-2184.2010.00674.x
30. Li F, Li X, Liu G, Gao C, Li X. Bone marrow mesenchymal stem cells decrease the expression of RANKL in collagen-induced arthritis rats via reducing the levels of IL-22. *J Immunol Res*. 2019;2019:8459281. doi:10.1155/2019/8459281
31. Oshita K, Yamaoka K, Udagawa N, et al. Human mesenchymal stem cells inhibit osteoclastogenesis through osteoprotegerin production. *Arthritis Rheum*. 2011;63(6):1658–1667. doi:10.1002/art.30309
32. Freitas GP, Lopes HB, Souza AT, et al. Cell terapy: Effect of locally injected mesenchymal stromal cells derived from bone marrow or adipose tissue on bone regeneration of rat calvarial defects. *Sci Rep*. 2019;9(1):13476. doi:10.1038/s41598-019-50067-6
33. Kim YH, Park M, Cho KA, et al. Tonsil-derived mesenchymal stem cells promote bone mineralization and reduce marrow and visceral adiposity in a mouse model of senile osteoporosis. *Stem Cells Dev*. 2016;25(15):1161–1171. doi:10.1089/scd.2016.0063
34. de Melo Ocarino N, Boeloni JN, Jorgetti V, Gomes DA, Goes AM, Serakides R. Intra-bone marrow injection of mesenchymal stem cells improves the femur bone mass of osteoporotic female rats. *Connect Tissue Res*. 2010;51(6):426–433. doi:10.3109/03008201003597049

Evaluation of new bioceramic endodontic sealers: An in vitro study

Rania El-Saady Badawy[1,B–F], Dalia AbdAllah Mohamed[2,A–F]

[1] Department of Biomaterials, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt
[2] Department of Endodontics, Faculty of Dentistry, Suez Canal Univeristy, Ismailia, Egypt

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):85–92

Address for correspondence
Rania El-Saady Badawy
E-mail: rania_badawy@dent.suez.edu.eg

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on December 27, 2020
Reviewed on March 1, 2021
Accepted on March 4, 2021

Published online on March 4, 2022

Abstract

Background. The biophysical properties of root canal sealers (RCSs) positively affect the success of endodontic treatment. It is important to ensure an impermeable apical seal after the thorough eradication of the infection. Since bioceramic sealers release bioactive and concomitantly biocompatible products after setting, chemical bonding to dentin and favorable healing is achieved.

Objectives. This study evaluated the chemical composition and elemental distribution of 4 RCSs (1 resin-based and 3 bioceramic-based) by using energy dispersive X-ray spectroscopy (EDX), field emission scanning electron microscopy (FE-SEM) and elemental mapping after root canal obturation, both coronally and apically.

Material and methods. Forty extracted single-rooted teeth were shaped, cleaned and randomly divided into 4 groups according to the type of sealer used for obturation. After the sealer set, the teeth were sectioned horizontally to obtain coronal and apical standardized sections. The sections were qualitatively and quantitatively assessed in terms of chemical composition of the tested sealers, using SEM images and elemental mapping as well as the EDX analysis.

Results. All of the calcium silicate sealers showed significantly higher peaks of calcium at the periphery of the root canals, contacting dentinal moisture, and high peaks of zirconium, while tungsten was significantly high in AH Plus™. TotalFill® BC™ and BioRoot™ RCS showed higher calcium, oxygen and silicon content coronally than apically, while phosphorus was only detected more apically, which was different for EndoSeal® MTA. All sealers revealed small amounts of different heavy metals, not described by their manufacturers, and a uniform particle distribution with almost regular surfaces.

Conclusions. All of the tested sealers except AH Plus revealed high calcium/phosphorus ratio peaks, suggesting regenerative potential in vivo, with acceptable purity and surface texture, and supporting their biocompatibility, with chemical bonding to root dentin.

Keywords: bioceramic, root canal sealers, energy dispersive X-ray spectroscopy, scanning electron microscopy, heavy metals

Cite as
Badawy RE, Mohamed DA. Evaluation of new bioceramic endodontic sealers: An in vitro study. *Dent Med Probl.* 2022;59(1):85–92. doi:10.17219/dmp/133954

DOI
10.17219/dmp/133954

Introduction

An adequate impermeable three-dimensional (3D) seal of the thoroughly disinfected root canal system is traditionally achieved using a gutta-percha core material and accessories cemented with a root canal sealer (RCS). Different types of sealers, including zinc oxide–eugenol (ZnO/E), calcium hydroxide ($CaOH_2$), glass ionomer (GI), silicone, epoxy resin or methacrylate resin, and bioceramic-based sealers, have been introduced into the market. These sealers differ in terms of their main chemical composition.[1]

Cytotoxicity and the absence of bonding to dentin, as well as the absence of hard tissue deposition, have been detected with ZnO/E sealers.[2] To overcome these shortcomings, resin-based sealers were introduced.[3] AH Plus™, the most widely used epoxy resin-based sealer, revealed good sealing ability, lower solubility in tissue fluids and minimal cytotoxic reactions, but no bioactivity with the surrounding tissues.[3]

The single-cone obturation method has been reintroduced to decrease the time, effort and extra forces required when using the lateral compaction technique. However, then, lower bond strength and adaptation to root canal dentin were observed. Hence, there is great demand to achieve the 'monoblock concept' through bonding to the core material and the dentinal walls, thus improving bond strength and adaptation.[4]

Since the 1970s, the application of ceramics in biomedicine has greatly expanded[5,6]; they can be either bioactive or bioinert in nature, depending on their interaction with vital tissues. Unlike bioinert ceramics, bioactive materials (bioactive glass, calcium silicates (Ca_2SiO_4) and calcium phosphates ($Ca_3(PO_4)_2$) interact with the surrounding tissues to encourage the growth and regeneration of more durable mineralized tissues.[1,5,7]

Hydraulic calcium silicates, or a combination of Ca_2SiO_4 and $Ca_3(PO_4)_2$ have been used as bioceramic-based sealers. They show unique bioactivity, as they set and harden in the presence of moisture, finally forming hydroxyapatite at the interface and creating a bond to dentin. These can be mineral trioxide aggregate (MTA)-based, non-MTA-based or calcium phosphate-based types.[1,6] In the presence of moisture, phosphate ($(PO_4)_2^{3-}$) partially reacts with Ca_2SiO_4 hydrogel and $CaOH_2$ to form hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) along the mineral infiltration zone.[1] The excellent biocompatibility of bioceramic sealers is most likely due to their similarity to biological $Ca_{10}(PO_4)_6(OH)_2$, as well as the presence of $Ca_3(PO_4)_2$, the main inorganic component of hard tissues. Bioceramic-based sealers have been observed to promote bone regeneration when unintentionally extruded periapically during root canal filling.[8]

The chemical composition of sealers determines their biophysical properties, which influence the formation of a hermetic seal, thus promoting the healing and regeneration of periapical tissues.[9]

Therefore, the aim of this study was to use energy dispersive X-ray spectroscopy (EDX) and field emission scanning electron microscopy (FE-SEM) to identify and characterize chemical elements and their distribution in AH Plus, EndoSeal® MTA, TotalFill® BC™, and BioRoot™ RCS sealers, along with comparing their bioactive and heavy metal content.

Material and methods

The chemical composition of the 4 different endodontic sealers is presented in Table 1. Forty sound, freshly extracted, single-rooted, single-canaled teeth with complete apices were used in this study (Fig. 1A).

Prior to sample preparation, the teeth were disinfected through immersion in 2.5% sodium hypochlorite solution (NaOCl) for 2 h, rinsed, and finally stored in saline solution.

The crowns were cut perpendicular to the long axis of the teeth with a diamond disk mounted on a slow-speed handpiece with a coolant, obtaining a root length

Table 1. Description of the materials used in the present study

Material	Type	Composition	Batch/ Manufacturer
AH Plus	epoxy resin-based sealer	Paste A (epoxide paste): diepoxide; calcium tungstate; zirconium oxide; iron oxide; and pigments Paste B (amine paste): 1-adamantanamine; N,N'-dibenzyl-5-oxanonandiamine-1,9; TCD-diamine; calcium tungstate; zirconium oxide; aerosil; and silicon oil	1705000906 Dentsply De Trey, Konstanz, Germany
EndoSeal MTA	bioceramic (mineral trioxide aggregate) sealer	Premixed syringe contains: calcium silicate; calcium aluminates; calcium sulfate; radiopacifier (zirconium oxide); bismuth oxide; thickening agent (hydroxypropyl methylcellulose); and N-methyl-2-pyrrolidone solvent	CI 171027A Maruchi, Wonju, South Korea
TotalFill BC	bioceramic sealer	Premixed syringe contains: zirconium oxide; calcium silicate; calcium phosphate; monobasic calcium hydroxide; fillers; and thickening agents	160045P FKG Dentaire, La Chaux-de-Fonds, Switzerland
BioRoot RCS	bioceramic sealer	Powder: tricalcium silicate; opacifier (zirconium oxide); and povidone (hydrophilic biocompatible polymer) Liquid: water; calcium chloride; and polycarboxylate (water-reducing agent)	B20645 Septodont, Saint-Maur-des-Fossés, Cedex – France

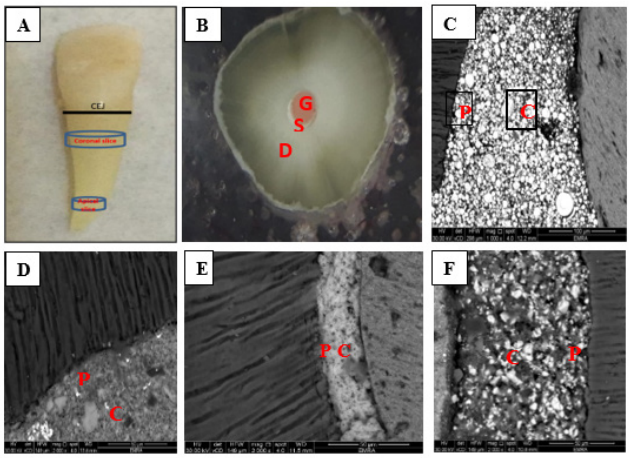

Fig. 1. A – photograph showing the sites of the planned apical and coronal slices (each of a thickness of 2 mm) below the cemento-enamel junction (CEJ), used for the energy dispersive X-ray spectroscopy (EDX) and field emission scanning electron microscopy (FE-SEM) assessments; B – photograph of the sliced root portion, showing the master cone (G), the sealer (S) and dentin (D); C–F – SEM images with the planned areas of the EDX analysis at the core (C) and periphery (P) of different sealers – AH Plus, EndoSeal MTA, TotalFill BC, and BioRoot RCS, respectively

of 15 ±1 mm. A K-file size 10 (Mani Inc., Takanezawa, Japan) was introduced to ensure the patency of the root canal and the working length of the canal was determined with a K-file size 15 (Mani Inc.). Each canal was irrigated with 5 mL of 2.5% NaOCl and instrumented up to size 20. An R40 reciprocal file (tip size 40 and a taper of 0.06 mm, VDW.SILVER® RECIPROC® endomotor; VDW, Munich, Germany) was used according to the manufacturer's instructions with a reciprocal motion to ensure a uniform internal width of the canal lumen.

A total of 5 mL of 2.5% NaOCl was used between the instrumentation steps, followed by 17% ethylenediaminetetraacetic acid (EDTA) (Prevest DenPro, Bari Brahmana, India) to remove the smear layer, and finally the canals were irrigated with 3 mL of 9% saline solution. The canals were gently dried using a dental ENDO Aspirator® root canal tip (Cerkamed, Stalowa Wola, Poland).

The teeth were randomly and equally divided into 4 groups according to the sealer used (n = 10 per group): AH Plus (group 1); EndoSeal MTA (group 2); TotalFill BC (group 3); and BioRoot RCS (group 4).

Each sealer was mixed and introduced into the canals according to the manufacturers' instructions; for AH Plus, additional dryness was achieved using matched paper points size 40. For obturation, a gutta-percha cone (tip size 40 and a taper of 0.06 mm) was dipped into the corresponding sealer, and then inserted and pressed to the full working length by means of a carrier. Excess material was removed with a heat carrier by applying a vertical compaction motion. After initial setting, the cavity was finally sealed with a GI filling material (Medicem; Promedica Dental Material, Neumünster, Germany). The teeth were placed on Petri dishes and stored in an incubator (37°C at 100% relative humidity) for 7 days.

Next, the teeth were placed into auto-polymerizing methyl methacrylate acrylic resin blocks. The roots were sectioned horizontally at the coronal (10 mm from the apex) and apical (4 mm from the apex) levels to obtain 2-millimeter-thick coronal and apical slices (Fig. 1A,1B).

The surfaces of both coronal and apical slices were examined at the core and periphery of the set sealers by using FE-SEM (model Quanta 250 FEG, Field Emission Gun; FEI Europe, Eindhoven, the Netherlands) with an accelerating voltage of 30 kV, magnification of ×14 up to ×2,000, a resolution of 132 eV, and an amplification time of 12.8 µs under vacuum. The chemical composition and elemental distribution of the sealers were determined using an EDX unit with the Noran System SIX (NSS) spectral analysis software, v. 2.3 (Thermo Fisher Scientific, Suwanee, USA) in the non-standard analysis mode (for automatic elemental identification) and with the phi-rho-Z (PROZA) correction. A dimensionally standardized rectangular area of analysis was set for all samples at the central and peripheral regions of the apical and coronal slices (Fig. 1C–1F).

Elemental maps were built according to the Net Counts Method microanalysis[10] at high resolution, showing surface regularity, elemental distribution, and the particle size and shape. The results were evaluated qualitatively, based on FE-SEM images and elemental mapping, and quantitatively, taking into account the element weight (wt%) and atomic (at%) percentages recorded during the EDX analysis. The particle size was measured using the ImageJ software (ImageJ 1.52d, Wayne Rasband, National Institutes of Health, USA). The scale measurements were calibrated for every image, marking the area of the particle size measurement, with differentiation from the surrounding area. The color threshold was adjusted, and the area and diameter of the distinguished particles were measured.

Statistical analysis

The data was statistically analyzed and tested for normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The one-way and two-way analyses of variance (ANOVA) were performed to evaluate the effect of the sealer type and its position within the root canal on the activity of the different elements available. This was followed by multiple comparisons using Duncan's post hoc test (α = 0.05). The IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA), was used for statistical analysis.

Results

The EDX spectral microanalysis and elemental mapping for the tested sealers revealed different elemental composition and distribution in terms of bioactive and heavy metal content expressed as wt% and at% (Table 2, Fig. 2–5).

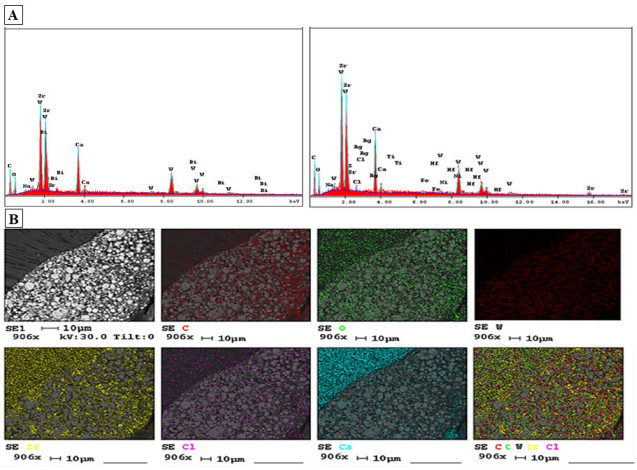

Fig. 2. A – EDX spectral microanalysis of AH Plus at magnification ×2,000 revealed primarily the highest peaks of W and Zr, with comparably lower peaks of C, O and Ca; furthermore, the material revealed minute peaks of Hf, Cl, Ti, Ni, and Fe; B – SEM image and elemental mapping of AH Plus at magnification ×906 showed C (reddish), O (green), W (brown-red), Zr (yellow), Cl (violet), and Ca (blue) uniformly distributed on the surface

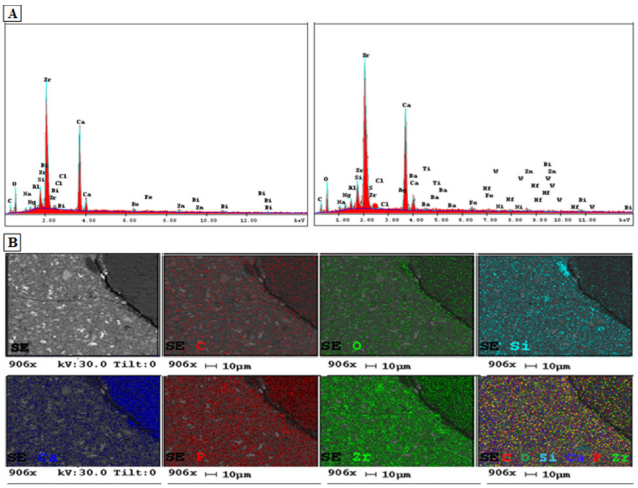

Fig. 3. A – EDX spectral microanalysis of EndoSeal MTA at magnification ×2,000 revealed higher peaks of Zr, Ca, and O, as well as lower peaks of C, Bi and Si; minute amounts of Mg, Na, Al, Ti, Hf, Ni, Zn, W, and Fe were also detected; B – SEM image and elemental mapping of EndoSeal MTA at magnification ×906 showed a uniform distribution of C (faint red), O (green), Si (blue), Ca (navy blue), P (red), and Zr (fluorescent green)

At the apical level, the EDX analysis showed that the highest calcium (Ca) wt% values were recorded for BioRoot RCS (19.22 ±0.5) and Endoseal MTA (19.32±0.6), followed by TotalFill BC (12.77 ±0.5). The highest carbon (C) wt% values were recorded for AH Plus (25.95 ±0.2), followed by BioRoot RCS (18.94 ±0.3), TotalFill BC (18.09 ±0.5) and EndoSeal MTA (11.81 ±0.5) (Table 2, Fig. 2A,3A,4A,5A).

Table 2. Concentration of elements expressed as weight (wt%) and atomic (at%) percentages in the tested root canal sealers (RCSs) at the apical level

Element	AH Plus		EndoSeal MTA		TotalFill BC		BioRoot RCS		*p*-value
	wt%	at%	wt%	at%	wt%	at%	wt%	at%	
Ca†	10.06 ± 0.4	6.70 ±0.4	19.32 ±0.6	12.31 ±0.5	12.77 ±0.5	8.49 ±0.3	19.22 ±0.5	11.24 ±0.8	≤0.001*
C†	25.95 ±0.2	58.44 ±0.5	11.81 ±0.5	25.71 ±0.4	18.09 ±0.5	39.55 ±0.3	18.94 ±0.3	38.27 ±0.2	≤0.001*
O†	15.04 ±0.7	25.01 ±0.7	26.02 ±1.0	43.74 ±0.6	22.70 ±0.8	36.06 ±0.4	24.20 ±1.2	38.07 ±1.1	≤0.001*
Si†	1.21 ±0.3	1.09 ±0.1	3.57 ±0.2	3.42 ±0.4	2.94 ±0.3	2.64 ±0.3	2.58 ±0.4	2.66 ±0.3	≤0.001*
P†	0	0	1.25 ±0.4	1.15 ±0.0	2.88 ±0.1	2.56 ±0.1	1.97 ±0.7	1.76 ±0.0	≤0.001*
Ca/P	0	0	15.46 ±2.9	10.31 ±3.3	4.43 ±0.5	3.32 ±0.3	9.75 ±2.6	6.38 ±2.8	≤0.001*
Zr#	12.88 ±0.6	3.77 ±0.4	26.37 ±1.0	7.72 ±0.2	38.64 ±0.7	10.91 ±0.4	27.10 ±0.6	7.37 ±0.4	≤0.001*
Bi#	0.34 ±0.0	0.05 ±0.0	6.59 ±0.4	0.88 ±0.1	1.53 ±0.2	0.19 ±0.0	1.71 ±0.1	0.19 ±0.0	≤0.001*
Cl	0.41 ±0.3	0.12 ±0.2	0.04 ±0.1	0.07 ±0.3	0.01 ±0.0	0.02 ±0.0	0.01 ±0.0	0.01 ±0.0	≤0.001*
Ti#	0.31 ±0.1	0.18 ±0.0	0.24 ±0.1	0.15 ±0.0	0.11 ±0.0	0.05 ±0.0	0.00 ±0.0	0.02 ±0.0	≤0.001*
W#	33.76 ±0.5	4.99 ±0.1	0.81 ±0.2	0.12 ±0.0	0.93 ±0.1	0.14 ±0.0	0.91 ±0.1	0.12 ±0.0	≤0.001*
Hf#	1.66 ±0.2	0.29 ±0.2	1.46 ±0.3	0.28 ±0.2	2.63 ±0.1	0.38 ±0.0	2.08 ±0.0	0.32 ±0.1	≤0.001*
S	0.02 ±0.0	0.01 ±0.0	0.18 ±0.0	0.29 ±0.2	0.07 ±0.1	0.06 ±0.1	0.17 ±0.3	0.19 ±0.3	≤0.001*
Ag#	0.01 ±0.0	0.01 ±0.0	0.01 ±0.0	0	0	0	0.03 ±0.0	0.02 ±0.0	≥0.05
Ba#	0	0	0.38 ±0.3	0.09 ±0.0	0	0	0.35 ±0.3	0.06 ±0.0	≤0.001*
Fe#	0.45 ±0.1	0.25 ±0.0	1.33 ±0.1	0.67 ±0.1	0.43 ±0.1	0.25 ±0.1	0.26 ±0.0	0.12 ±0.0	≤0.001*
Ni#	0.71 ±0.1	0.32 ±0.1	0.24 ±0.1	0.11 ±0.0	0.54 ±0.2	0.25 ±0.1	0.35 ±0.2	0.13 ±0.0	≤0.001*
Zn#	0	0	1.45 ±0.6	0.62 ±0.1	1.92 ±0.5	0.76 ±0.1	5.81 ±1.2	2.13 ±0.1	≤0.001*
Mg	0	0	1.23 ±0.1	1.31 ±0.0	0.37 ±0.1	0.39 ±0.0	0.63 ±0.3	0.62 ±0.0	≤0.001*
Al#	0	0	1.59 ±0.1	1.63 ±0.1	0.17 ±0.1	0.16 ±0.0	0	0	≤0.001*
Na	0.78 ±0.0	0.85 ±0.0	1.67 ±0.1	1.90 ±0.0	0.32 ±0.1	0.35 ±0.0	0.95 ±0.3	1.06 ±0.0	≤0.001*

Data presented as mean ± standard deviation (*M ±SD*). Ca – calcium; C – carbon; O – oxygen; Si – silicon; P – phosphorus; Ca/P – calcium/phosphorus ratio; Zr – zirconium; Bi – bismuth; Cl – chlorine; Ti – titanium; W – tungsten; Hf – hafnium; S – sulfur; Ag – silver; Ba – barium; Fe – iron; Ni – nickel; Zn – zinc; Mg – magnesium; Al – aluminum; Na – sodium; † bioactive element; # heavy metal; * statistically significant.

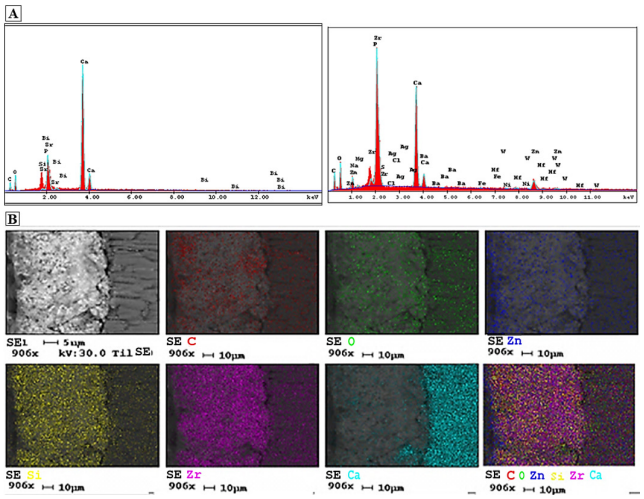

Fig. 4. A – EDX spectral microanalysis of TotalFill BC at magnification ×2,000 revealed higher peaks of Ca, Zr and P, with lower peaks of C, O, Zn, and Si; minute peaks of Na, W, Bi, and Cl were also detected; B – SEM image and elemental mapping of TotalFill BC at magnification ×906 showed a uniform distribution of C (greyish-red), O (green), Zn (navy blue), Si (yellow), Zr (violet), and Ca (blue)

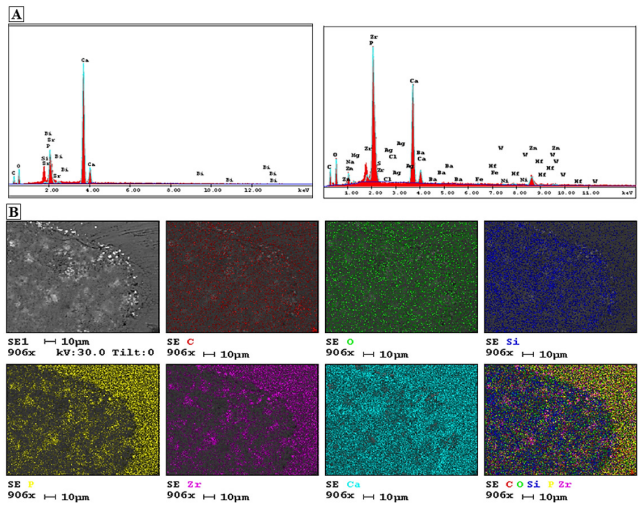

Fig. 5. A – EDX spectral microanalysis of BioRoot RCS at magnification ×2,000 revealed higher peaks for Ca and Zr, with lower peaks of O, C, Si, Zn, Bi, and P; minute peaks of Hf, Ni, Ba, Fe, and Cl were also detected; B – SEM image and elemental mapping of BioRoot RCS at magnification ×906 showed a uniform distribution of prominent Ca (blue), in addition to C (red), O (green), Si (navy blue), P (greenish-yellow), and Zr (violet)

Higher silicon (Si) wt% values were found in EndoSeal MTA (3.57 ±0.2), followed by TotalFill BC (2.94 ±0.3) and BioRoot RCS (2.58 ±0.4). EndoSeal MTA presented the highest oxygen (O) and calcium/phosphorus ratio (Ca/P) wt% values (26.02 ±1.0 and 15.46 ±2.9, respectively), followed by BioRoot RCS (24.20 ±1.2 and 9.75 ±2.6, respectively) and TotalFill BC (22.70 ±0.8 and 4.43 ±0.5, respectively). A more significant wt% value of phosphorus (P) was detected in TotalFill BC (2.88 ±0.1) than in BioRoot RCS (1.97 ±0.7) and EndoSeal MTA (1.25 ±0.4) (Table 2, Fig. 2A,3A,4A,5A).

All the tested sealers revealed the content of heavy metals (zirconium (Zr), bismuth (Bi), tungsten (W), hafnium (Hf), iron (Fe), and nickel (Ni)) at significantly different proportions, with Zr being the most prominent. TotalFill BC showed the highest Zr wt% value, followed by BioRoot RCS, EndoSeal MTA, and AH Plus. Tungsten was the most prominent heavy metal in terms of wt% in the AH Plus sealer, while the wt% value of Bi was significantly higher in EndoSeal MTA than in the other tested sealers. Iron and Ni were found in small amounts in all the tested sealers (Table 2).

Traces of other heavy metals (titanium (Ti), silver (Ag), barium (Ba), zinc (Zn), and aluminum (Al)) were present in some of the tested sealers, while others showed their absence. Titanium was absent in BioRoot RCS, Ag was absent in TotalFill BC, Ba was absent in AH Plus and TotalFill BC, and Al was absent in AH Plus and BioRoot RCS. Zinc was absent in AH Plus, but significantly prominent in BioRoot RCS. Traces of chlorine (Cl), sulfur (S), magnesium (Mg), and sodium (Na) were also detected in all sealers except AH Plus, where Mg was absent (Table 2).

The comparison of the wt% values of bioactive elements in the different sealers tested at different tooth levels (the coronal core, the coronal periphery, the apical core, and the apical periphery) showed statistically significant differences in the concentration of nearly all bioactive elements at various root canal levels, except for AH Plus, which showed the complete absence of P, and thus no Ca/P, the lowest concentration of Ca, O and Si, but the highest concentration of C at all root canal levels (Table 3).

For all calcium silicate sealers, significantly higher peaks of Ca were detected at the periphery of the root canals as compared to their core, but Si revealed a higher concentration at the core than at the periphery. For TotalFill BC and BioRoot RCS, the Ca, O and Si content was higher at the coronal portion, while the P content was higher more apically. These results were reversed for EndoSeal MTA (Table 3).

The highest Ca/P ratio for EndoSeal MTA (15.46 ±2.9) was recorded at the apical peripheral root level, while the highest values for TotalFill BC (8.69 ±3.1) and BioRoot RCS (34.25 ±4.8) were recorded at the coronal peripheral root level. (Table 3).

The SEM images of the AH Plus specimens revealed regular surfaces with globular-like particles (mean area of 6.1 ±11 μm^2 and diameter of 2.1 ±1.8 μm) (Fig. 1C,2B). The EndoSeal MTA specimens showed slightly irregular crystalline surfaces, including particles of different shapes (needle-like, globular-like and matrix-like) and sizes (mean area of 2.6 ±7.9 μm^2 and diameter of 1.2 ±1.4 μm) (Fig. 1D,3B). TotalFill BC revealed regular surfaces with homogenous, smaller and matrix-like particles (mean area of 0.6 ±1.4 μm^2 and diameter of 0.6 ±0.6 μm) (Fig. 1E,4B). The SEM analysis of the BioRoot RCS surface revealed regular globular-like particles embedded in a similarly shaped matrix (mean area of 5.5 ±12.2 μm^2 and diameter of 1.9 ±1.8 μm) (Fig. 1F,5B).

Table 3. Concentration of bioactive elements expressed as weight percentage (wt%) at the core and periphery of both coronal and apical levels for the tested root canal sealers (RCSs)

Element	Slice area	AH Plus	*p*-value	EndoSeal MTA	*p*-value	TotalFill BC	*p*-value	BioRoot RCS	*p*-value
Ca	Cc	9.57^{a} ±0.3	≥0.05	15.01^{c} ±0.5	≤0.05*	11.43^{b} ±4.2	≤0.05*	33.65^{b} ±7.1	≤0.05*
	Cp	10.63^{a} ±0.5		17.13^{b} ±0.7		16.69^{a} ±4.8		39.04^{a} ±1.2	
	Ac	10.49^{a} ±0.6		17.11^{b} ±0.6		11.03^{b} ±0.7		16.50^{c} ±0.7	
	Ap	10.06^{a} ±0.4		19.32^{a} ±0.6		12.77^{b} ±0.5		19.22^{c} ±0.5	
C	Cc	27.70^{a} ±3.7	≤0.05*	11.40^{b} ±0.9	≤0.05*	26.12^{a} ±1.9	≤0.05*	12.21^{c} ±0.5	≤0.05*
	Cp	26.95^{a} ±3.4		12.41^{a} ±2.7		17.07^{c} ±3.3		12.90^{b} ±0.5	
	Ac	22.54^{c} ±0.5		12.12^{a} ±0.4		19.47^{b} ±0.6		19.04^{a} ±0.4	
	Ap	25.95^{b} ±0.2		11.81^{b} ±0.5		18.09^{c} ±0.5		18.94^{a} ±0.3	
O	Cc	12.46^{b} ±1.3	≤0.05*	25.74^{b} ±1.0	≤0.05*	24.88^{b} ±3.2	≤0.05*	32.92^{a} ±3.5	≤0.05*
	Cp	12.85^{b} ±1.2		26.09^{b} ±1.5		30.72^{a} ±1.7		32.01^{a} ±1.4	
	Ac	13.32^{b} ±0.3		28.05^{a} ±0.6		24.35^{c} ±0.7		25.39^{b} ±0.1	
	Ap	15.04^{a} ±0.7		26.02^{b} ±1.0		22.70^{c} ±0.8		24.20^{b} ±1.2	
Si	Cc	1.40^{a} ± 0.0	≤0.05*	4.12^{a} ±0.2	≤0.05*	5.99^{a} ±1.4	≤0.05*	5.12^{a} ±1.8	≤0.05*
	Cp	1.41^{a} ±0.2		3.45^{b} ±0.7		4.53^{b} ±0.5		2.89^{c} ±0.5	
	Ac	1.33^{b} ±0.3		4.52^{a} ±0.3		3.99^{b} ±0.3		4.41^{b} ±0.4	
	Ap	1.21^{c} ±0.3		3.57^{b} ±0.2		2.94^{c} ±0.3		2.58^{c} ±0.3	
P	Cc	0^{a}	≥0.05	1.45^{a} ±0.2	≤0.05*	1.48^{c} ±0.2	≤0.05*	1.76^{a} ±0.3	≤0.05*
	Cp	0^{a}		1.46^{a} ±0.3		1.92^{b} ±0.1		1.14^{c} ±0.1	
	Ac	0^{a}		1.23^{b} ±0.1		1.67^{c} ±0.1		1.44^{b} ±0.0	
	Ap	0^{a}		1.25^{b} ±0.0		2.88^{a} ±0.1		1.97^{a} ±0.0	
Ca/P	Cc	0^{a}	≥0.05	10.35^{b} ±3.0	≤0.05*	7.72^{b} ±2.1	≤0.05*	19.11^{b} ±4.2	≤0.05*
	Cp	0^{a}		11.73^{b} ±2.3		8.69^{a} ±3.1		34.25^{a} ±4.8	
	Ac	0^{a}		13.91^{a} ±3.3		6.60^{bc} ±1.4		11.45^{b} ±3.5	
	Ap	0^{a}		15.46^{a} ±2.9		4.43^{c} ±0.5		9.75^{c} ±2.6	

Data presented as *M* ±*SD*. Cc – coronal core; Cp – coronal periphery; Ac – apical core; Ap – apical periphery; * statistically significant. Different uppercase letters denote statistically significant differences between the w% of particular elements in various sealers at various root canal levels ($p < 0.05$).

Discussion

To control the quality of endodontic materials, their tissue tolerance and interaction with the tooth structure should be tested. Many standardized technological tests according to the American National Standards Institute/American Dental Association (ANSI/ADA) in the USA and the International Organization for Standardization (ISO) internationally have been applied for this purpose. Such tests include the combined EDX microanalysis and SEM elemental mapping analysis, which describe all trace elements and the surfaces of chemical elements quantitatively and qualitatively.[7,9,10]

X-ray spectrometry is not capable of distinguishing between ionic and non-ionic types of elements, and it also has a detection limit of ~0.1%, which makes the detection of light elements sometimes inaccurate, depending on the element itself.[9] Field emission scanning electron microscopy was used in this study, as it neglects the surface treatment or manipulation step prior to analysis. Accordingly, low-energy X-rays, which are characteristic of light elements, were not absorbed in the gold surface layer, as was observed in previous studies.[9,11] Also, X-ray detection is not affected by the chemical state of elements, but it is influenced by inter-element interference, known in X-ray spectrometry as the peak overlap. This may cause serious problems in the analysis of elements with similar energy peaks, such as W and Si in AH Plus.[9] As a trial to overcome this problem, this study used net counts, which are representative of the chemical element of each energy peak, and also provide an accurate analysis of the chemical composition of the material. Accordingly, some authors suggest some additional analysis to SEM-EDX, such as atomic absorption spectrometry (AAS), X-ray fluorescence (XRF), X-ray diffraction analysis (XRD), and inductively coupled plasma optical emission spectrometry (ICP-OES), to increase the accuracy of elemental characterization.[12]

AH Plus, an epoxy resin-based sealer, was used in this study as a reference, as it is considered to be the gold standard in terms of sealing ability and low solubility, according to the literature.

Al-Haddad et al. stated that the chemical composition of the set bioceramic sealers varied according to the surrounding environment during the setting reaction.[13] Thus, it was important to simulate natural oral conditions by conducting the study on freshly extracted teeth[13] instead of using cylindrical molds or polyethylene tubes, which

were commonly employed in different studies.[9,11,14,15] Thereby, the effect of microstructure and dentinal moisture at different tooth levels on the bioactivity of the tested sealers and their clinical significance in terms of bonding to dentin and sealing ability could be evaluated.[11,14]

Unlike in the case of the AH Plus sealer, the concentration (wt%) of bioactive elements in the tested bioceramic sealers varied significantly at different root canal levels. The TotalFill BC and BioRoot RCS sealers revealed a higher concentration of bioactive elements (Ca, O and Si) coronally. This may be due to the larger diameter of dentinal tubules coronally (4.32 µm), allowing more sealer penetration and greater moisture contact than in the middle (3.74 µm) and apical (1.73 µm) zones.[16,17]

Due to the modified setting reaction,[18] the resultant change in the composition of the bioactive products occurred with different levels of hydration.[13,19]

The difficult removal of the smear layer at the apical third might also act as a physical barrier, interfering with the sealer adaptation to root canal dentin moisture.[20]

EndoSeal MTA showed different bioactive element distribution at variable root levels as compared to the other sealers, which may be due to differences in the flow and the setting time, making it less sensitive to changes in dentin moisture.[20]

The higher Ca/P ratios detected at the peripheral root portions confirm the effect of dentin moisture on the bioactivity of bioceramic sealers through the complex hydration reaction.

The Ca/P atomic ratios detected in this study for BioRoot RCS and TotalFill BC (6.38 ±2.8 at% and 3.32 ±0.3 at%, respectively) are more or less in agreement with those found in BioRoot RCS (3.20–5.21 at%) by Siboni et al.[21] However, the EndoSeal MTA Ca/P atomic ratio (10.70 ±3.3 at%) is not in accordance with those obtained by Yoo et al. (1.45–1.89 at%).[22]

Unfortunately, the Ca/P ratios for all the bioceramic sealers tested in the present study contradicted the manufacturers' claims, being different from those of the tooth structure (1.5–1.67 wt%).[23] Such differences might be the result of not using phosphate buffered saline (PBS) or Hank's Balanced Salt Solution (HBSS) in any treatment or storage, as these are quite important for the formation of a mineralized apatite structure.[5,13,21,22]

In the present study, a lower presentation of P wt% was observed for the EndoSeal MTA, TotalFill BC and BioRoot RCS samples, which explains an increase in the Ca/P ratio.[5]

Due to the complete absence of P and Ca/P, and the lowest Ca concentration, AH Plus revealed a low capacity of inducing periapical repair as compared to the rest of the endodontic sealers that were investigated.[6,24] This is consistent with the findings of Sampaio et al.[9] and Reszka et al.[11] However, Siboni et al. detected a thin Ca/P deposit on AH Plus immersed in HBSS.[21]

All the tested sealers showed regular surfaces with uniformly distributed particles that were similar in shape, but different in size, except for EndoSeal MTA, which showed different particle shapes and slightly irregular surfaces.

According to Balto and Al-Nazhan[25] and Sampaio et al.,[9] better cell adhesion and biocompatibility are expected with the AH Plus, TotalFill BC and BioRoot RCS sealers. However, EndoSeal MTA showed more cell adhesion and viability than AH Plus in other cytological and histological studies,[26] which emphasizes the impact of other factors, such as bioactivity and heavy metal toxicity, on biological behavior.

Based on the literature, AH Plus has cytotoxic effects due to its epoxy resin content and the release of toxic monomers, such as bisphenol A diglycidyl ether, which delays periapical healing when extruded apically.[6,24]

Due to the clinical importance of the direct and indirect contact of the sealer with periapical tissues, elemental composition and concentration in the tested sealers were compared at the apical level. Consistent with the literature, dissimilarity was detected in the tested sealers at the apical level,[5,11,13,21] although Ca_2SiO_4 is the main component in the 3 bioceramic sealers tested.[19] This dissimilarity was due to the different sources of Ca and additives provided by their manufacturers, and their different presentation forms.

According to the literature, heavy elements (Zr, Bi, Ti, W, and Hf) have been added to sealers to increase their radiopacity. High peaks of Zr and low peaks of Hf were detected in all the tested sealers, as well as Bi in EndoSeal MTA and W in AH Plus (from calcium tungstate ($CaWO_4$)).[27] This explains the high radiopacity of AH Plus reported in the literature,[26,28] higher than in the case of BioRoot RCS,[21] followed by the EndoSeal MTA and EndoSequence® BC™ sealers.[28] Although zirconium dioxide (ZrO_2) provides a lower contrast than other radiopacifiers (W and bismuth oxide (Bi_2O_3)), it seems to be more inert,[7,19] allowing a longer release of calcium ions, thus making the tricalcium silicate (Ca_3SiO_5) cements more biocompatible.[13,29]

Several heavy metals (Bi, W, Hf, Ag, Ba, Ni, Zn, and Al) were detected in the tested sealers, which could possibly endanger periapical cells, and also affect tissue healing.

Some of the sealers showed certain elements that had not been mentioned by the manufacturers (Bi, Hf, Fe, Ni, Zn, Mg, and Al) in small wt%, either due to contamination during manufacturing or as industrial secrets.[29]

Differences in heavy metal composition between the cited literature[11,13,30] and the present study may be due to variations in the experimental conditions.

Further studies should be conducted to evaluate the effect of elemental composition on the biological and physicochemical features of calcium silicate-based sealers, aiming to minimize the harmful effects while enhancing endodontic repair.

Conclusions

When in contact with root dentin moisture, the tested bioceramic sealers released different percentages

of bioactive elements at the periphery, which could possibly enhance chemical bonding to root dentin. All the tested sealers except AH Plus revealed high peaks of the Ca/P ratio, suggesting regenerative potential in vivo, which supports their biocompatibility. The Ca/P ratio and heavy metal content were not in complete agreement with those suggested by the manufacturers.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Rania El-Saady Badawy https://orcid.org/0000-0002-9145-4520
Dalia AbdAllah Mohamed https://orcid.org/0000-0001-5724-9251

References

1. Al-Haddad A, Ab-Aziz ZAC. Bioceramic-based root canal sealers: A review. *Int J Biomater.* 2016;2016:9753210. doi:10.1155/2016/9753210
2. Rathi CH, Chandak M, Nikhade P, et al. Functions of root canal sealers – a review. *J Evolution Med Dent Sci.* 2020;9(17):1454–1458. doi:10.14260/jemds/2020/317
3. Okşan T, Aktener BO, Sen BH, Tezel H. The penetration of root canal sealers into dentinal tubules. A scanning electron microscopic study. *Int Endod J.* 1993;26(5):301–305. doi:10.1111/j.1365-2591.1993.tb00575.x
4. Mohamed El-Sayed MA, Aziz Taleb AA, Mubarak Balbahaith MS. Sealing ability of three single-cone obturation systems: An in-vitro glucose leakage study. *J Conserv Dent.* 2013;16(6):489–493. doi:10.4103/0972-0707.120936
5. Jafari F, Jafari S. Composition and physicochemical properties of calcium silicate based sealers: A review article. *J Clin Exp Dent.* 2017;9(10):e1249–e1255. doi:10.4317/jced.54103
6. Zhang W, Li Z, Peng B. Ex vivo cytotoxicity of a new calcium silicate-based canal filling material. *Int Endod J.* 2010;43(9):769–774. doi:10.1111/j.1365-2591.2010.01733.x
7. Camilleri J. Tricalcium silicate cements with resins and alternative radiopacifiers. *J Endod.* 2014;40(12):2030–2035. doi:10.1016/j.joen.2014.08.016
8. Bryan TE, Khechen K, Brackett MG, et al. In vitro osteogenic potential of an experimental calcium silicate-based root canal sealer. *J Endod.* 2010;36(7):1163–1169. doi:10.1016/j.joen.2010.03.034
9. Sampaio FC, Gonçalves Alencar AH, Guedes OA, Pinho Veloso HH, Santos TO, Estrela C. Chemical elements characterization of root canal sealers using scanning electron microscopy and energy dispersive X-ray analysis. *Oral Health Dent Manag.* 2014;13(1):27–34. PMID:24603912.
10. Newbury DE, Richi NW. Is scanning electron microscopy/energy dispersive X-ray spectrometry (SEM/EDS) quantitative? *Scanning.* 2013;35(3):141–168. doi:10.1002/sca.21041
11. Reszka P, Nowicka A, Lipski M, Dura W, Droździk A, Woźniak K. A comparative study of calcium silicate-containing and epoxy resin-based root canal sealers. *Biomed Res Int.* 2016;2016:9808432. doi:10.1155/2016/9808432
12. Estrela C, Sousa-Neto MD, Guedes OA, Gonçalves Alencar AH, Hungaro Duarte MA, Pécora JD. Characterization of calcium oxide in root perforation sealers materials. *Braz Dent J.* 2012;23(5):539–546. doi:10.1590/s0103-64402012000500012
13. Al-Haddad AY, Kutty MG, Abu Kasim NH, Ab Aziz ZA. The effect of moisture conditions on the constitution of two bioceramic-based root canal sealers. *J Dent Sci.* 2017;12(4):340–346. doi:10.1016/j.jds.2017.03.008
14. Borges ÁH, Guedes OA, Orçati Dorilêo MC, Bandeca MC, Almeida Estrela CR, Estrela C. Analysis of chemical elements and heavy metals in MTA Fillapex and AH Plus. *Oral Health Dent Manag.* 2014;13(4):1007–1012. http://repositorio.bc.ufg.br/handle/ri/16565. Accessed December 20, 2020.
15. Loushine BA, Bryan TE, Looney SW, et al. Setting properties and cytotoxicity evaluation of a premixed bioceramic root canal sealer. *J Endod.* 2011;37(5):673–677. doi:10.1016/j.joen.2011.01.003
16. Baroni Barbizam JV, Trope M, Tanomaru-Filho M, Nogueira Teixeira EC, Teixeira FB. Bond strength of different endodontic sealers to dentin: Push-out test. *J Appl Oral Sci.* 2011;19(6):644–647. doi:10.1590/S1678-77572011000600017
17. Lo Giudice G, Cutroneo G, Centofanti A, et al. Dentin morphology of root canal surface: A quantitative evaluation based on a scanning electronic microscopy study. *Biomed Res Int.* 2015;2015:164065. doi:10.1155/2015/164065
18. Zmener O, Pameijer CH, Serrano SA, Vidueira M, Macchi RL. Significance of moist root canal dentin with the use of methacrylate-based endodontic sealers: An in vitro coronal dye leakage study. *J Endod.* 2008;34(1):76–79. doi:10.1016/j.joen.2007.10.012
19. Gandolfi MG, Prati C. MTA and F-doped MTA cements used as sealers with warm gutta-percha. Long-term study of sealing ability. *Int Endod J.* 2010;43(10):889–901. doi:10.1111/j.1365-2591.2010.01763.x
20. Violich DR, Chandler NP. The smear layer in endodontics – a review. *Int Endod J.* 2010;43(1):2–15. doi:10.1111/j.1365-2591.2009.01627.x
21. Siboni F, Taddei P, Zamparini F, Prati C, Gandolfi MG. Properties of BioRoot RCS, a tricalcium silicate endodontic sealer modified with povidone and polycarboxylate. *Int Endod J.* 2017;50(Suppl 2):e120–e136. doi:10.1111/iej.12856
22. Yoo YJ, Baek SH, Kum KY, Shon WJ, Woo KM, Lee WC. Dynamic intratubular biomineralization following root canal obturation with pozzolan-based mineral trioxide aggregate sealer cement. *Scanning.* 2016;38(1):50–56. doi:10.1002/sca.21240
23. Rey C, Combes C, Drouet C, Grossin D. 1.111. Bioactive ceramics: Physical chemistry. In: Ducheyne P, Healy DE, Hutmacher DW, Grainger DW, Kirkpatrick CJ, eds. *Comprehensive Biomaterials*. 1st ed. Amsterdam, the Netherlands: Elsevier Science; 2011:187–221. doi:10.1016/B978-0-08-055294-1.00178-1
24. Lodienè G, Kopperud HM, Ørstavik D, Bruzell EM. Detection of leachables and cytotoxicity after exposure to methacrylate- and epoxy-based root canal sealers in vitro. *Eur J Oral Sci.* 2013;121(5):488–496. doi:10.1111/eos.12065
25. Balto H, Al-Nazhan S. Attachment of human periodontal ligament fibroblasts to 3 different root-end filling materials: Scanning electron microscope observation. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2003;95(2):222–227. doi:10.1067/moe.2003.96
26. Lim ES, Park YB, Kwon YS, Shon WJ, Lee KW, Min KS. Physical properties and biocompatibility of an injectable calcium-silicate-based root canal sealer: In vitro and in vivo study. *BMC Oral Health.* 2015;15(1):129. doi:10.1186/s12903-015-0112-9
27. Garrido AD, Lia RC, França SC, Da Silva JF, Astolfi-Filho S, Sousa-Neto MD. Laboratory evaluation of the physicochemical properties of a new root canal sealer based on *Copaifera multijuga* oil-resin. *Int Endod J.* 2010;43(4):283–291. doi:10.1111/j.1365-2591.2009.01678.x
28. Lee KJ, Kwak SW, Ha JH, Lee WC, Kim HC. Physicochemical properties of epoxy resin-based and bioceramic-based rootcanal sealers. *Bioinorg Chem Appl.* 2017;2017:2582849. doi:10.1155/2017/2582849
29. Li X, Yoshihara K, De Munck J, et al. Modified tricalcium silicate cement formulations with added zirconium oxide. *Clin Oral Investig.* 2017;21(3):895–905. doi:10.1007/s00784-016-1843-y
30. Viapiana R, Guerreiro-Tanomaru JM, Hungaro-Duarte MA, Tanomaru-Filho M, Camilleri J. Chemical characterization and bioactivity of epoxy resin and Portland cement-based sealers with niobium and zirconium oxide radiopacifiers. *Dent Mater.* 2014;30(9):1005–1020. doi:10.1016/j.dental.2014.05.007

Effect of statin therapy on oral *Candida* carriage in hyperlipidemia patients: A pioneer study

Azmi Darwazeh[1,A–F], Hisham Al-Shorman[2,A–F], Bayan Mrayan[3,A–F]

[1] Department of Oral Medicine and Surgery, Faculty of Dentistry, Jordan University of Science and Technology, Irbid, Jordan
[2] Department of Preventive Dentistry, Faculty of Dentistry, Jordan University of Science and Technology, Irbid, Jordan
[3] Postgraduate student, Department of Preventive Dentistry, Faculty of Dentistry, Jordan University of Science and Technology, Irbid, Jordan

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):93–97

Address for correspondence
Azmi Darwazeh
E-mail: darwazeh@just.edu.jo

Funding sources
The study was supported by a grant from the Deanship of Scientific Research at Jordan University of Science and Technology, Irbid, Jordan (grant No. 20180404).

Conflict of interest
None declared

Acknowledgements
The authors would like to thank the Deanship of Scientific Research at Jordan University of Science and Technology, Irbid, Jordan, for sponsoring this research.

Received on July 4, 2021
Reviewed on September 2, 2021
Accepted on September 28, 2021

Published online on February 28, 2022

Abstract

Background. Hyperlipidemia affects about 25% of the adult population globally. Statins are the most commonly used drugs in the management of hyperlipidemia. Laboratory and retrospective clinical studies have observed the inhibitory effects of statins on the growth of different *Candida* species. The effect of statin therapy on oral *Candida* carriage has not yet been investigated.

Objectives. This pioneer controlled study aimed to assess and compare asymptomatic oral *Candida* carriage in a group of 50 hyperlipidemic patients who were on regular statin therapy (HLS group) and in a control group of 50 subjects, matched in terms of gender, age and dental status, who were hyperlipidemic, but not on statin therapy (HLNS group).

Material and methods. The patients were recruited from the outpatient clinics of 2 university hospitals. The concentrated oral rinse technique was used to isolate oral *Candida* species in both groups. *Candida* species were identified using the germ tube test and the VITEK® 2 system.

Results. The *Candida* prevalence and colony count were significantly lower in the HLS group as compared to the HLNS group ($n = 20$, 40% vs. $n = 30$, 60%, respectively; $p = 0.040$). There was no significant difference in the oral *Candida* prevalence or colony count between different age groups in either the statin or control subjects.

Conclusions. Statin therapy is associated with a reduction in oral *Candida* carriage in both prevalence and the colony count in hyperlipidemic patients.

Keywords: *Candida albicans*, hyperlipidemia, statin therapy, oral *Candida*

Cite as
Darwazeh A, Al-Shorman H, Mrayan B. Effect of statin therapy on oral *Candida* carriage in hyperlipidemia patients: A pioneer study. *Dent Med Probl.* 2022;59(1):93–97. doi:10.17219/dmp/142641

DOI
10.17219/dmp/142641

Introduction

Elevated blood cholesterol (hypercholesterolemia) is a common metabolic disorder that affects about 25% of adults globally and 44.3% of adult Jordanians.[1] Statins, such as simvastatin and atorvastatin, are the most commonly prescribed medications to control hypercholesterolemia.[2] Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that interfere with the biosynthesis of cholesterol. The main antifungal action of polyenes (nystatin and amphotericin B) has been shown to be the destruction of ergosterol, a component of the fungal cell wall (the fungal counterpart of mammalian cell cholesterol), leading to the leakage of the fungal cell cytoplasm and cell death.[2]

Candida species are widely spreading opportunistic pathogens that constitute part of the oral ecology in up to 60% of normal individuals, with racial and geographic variations. They can cause superficial or, more seriously, hematogenous infections if the host immune barriers are breached, either on the local or systemic level.[3]

Reports have described several pleiotropic effects of statins apart from their serum cholesterol-lowering action, including anti-oxidative, anti-inflammatory, immunomodulatory, and antimicrobial properties.[4] A review of the literature revealed several studies describing the antifungal effects of statins.[5] Relevant to this, simvastatin inhibited the formation of the *Candida albicans* (*C. albicans*) biofilm in an in vitro study.[6] Several studies have shown the growth inhibitory effects of statins against different *Candida* species.[6–10] On the clinical side, a retrospective multicenter cohort study of 326 patients with candidemia reported candidemia in 13.5% of statin users as compared to 86.5% of statin non-users.[11] Another study included 1,019 diabetic patients who underwent lower gastrointestinal tract surgery; the patients who were on statin therapy had significantly fewer positive cultures for *Candida* species from the samples of blood, urine, sputum, and peritoneal fluid during hospitalization.[12] However, one animal study found that atorvastatin lowered the survival rate in a group of mice infected with *C. albicans* via peritoneal injection as compared to infected mice which were not treated with atorvastatin.[13] Welch et al. reviewed 124 candidemia episodes and concluded that statin use did not alter mortality, the length of hospital stay or intensive care requirement.[14]

To the authors' knowledge, no prospective clinical study has investigated the effect of statin therapy on the asymptomatic oral carriage of *Candida* species. The aims of this pioneer study were to assess and compare oral *Candida* species carriage, i.e., the positive oral isolation of *Candida* species without any clinical signs or symptoms of candidal infection, in a group of hypercholesterolemia patients who were on statin therapy and in a control group of hypercholesterolemia patients, matched in terms of gender, age and dental status, who were not on statin therapy.

Material and methods

Study subjects

The subjects of this study were recruited from the Internal Medicine Clinic at King Abdullah University Hospital (KAUH), the Family Medicine Clinic at Jordan University of Science and Technology Health Center and the Internal Medicine Outpatient Clinic at Princess Basmah Teaching Hospital in Irbid, Jordan, between February 2019 and July 2019. The subjects willingly agreed to participate in the study and signed a formal consent form. The study group was composed of 50 hyperlipidemic patients who were on regular statin therapy to control their hypercholesterolemia, in addition to dietary regime and physical activity, for at least 8 months; they were called the hyperlipidemia statin group (HLS group). The control group consisted of 50 gender-, age- and dental status-matched hyperlipidemic patients whose hyperlipidemia was managed only by diet control and physical exercise. They were not on statin therapy and were called the hyperlipidemia non-statin group (HLNS group). The minimal sample size for this pioneer study was determined after consultation with a biostatistician. Hyperlipidemic patients were identified based on previous serum cholesterol level investigations that were requested and confirmed by their attending physicians.

The inclusion criteria were as follows:
– adult patients diagnosed with hypercholesterolemia;
– non-smokers; and
– dentate subjects with at least 10 teeth.

The exclusion criteria were as follows:
– subjects with a removable dental prosthesis;
– tobacco smokers;
– diabetic or hypertensive subjects;
– subjects with complaints of xerostomia;
– subjects with a history of oral candidosis for the last 6 months;
– subjects who were on antibacterial, antifungal or immunosuppressant therapy;
– subjects who were using an antiseptic mouthwash regularly for the last 6 months; and
– subjects who exhibited mucosal changes suggestive of candidosis, clinically evident calculus or overt gingival inflammation.

The subjects in the HLS group were recruited into the study first. Then, the HLNS subjects who matched the corresponding subjects in the HLS group were included.

Microbiological investigations

The concentrated oral rinse technique described by Samaranayake et al. was used for quantitative and qualitative oral *Candida* isolation.[15] Briefly, each subject was supplied with 10 mL of 0.9% sterile normal saline

in a universal container. They were asked to rinse their mouth thoroughly with the saline solution for one full minute, and then return the rinse into the container. The samples were transported immediately in an icebox to the microbiology laboratory at KAUH for *Candida* species identification. The mouth rinse was centrifuged at 1,700 g for 15 min and the supernatant was discarded. The deposit was reconstituted in 1 mL of 0.9% sterile normal saline and agitated with a vortex mixer (Assistent®; Glaswarenfabrik Karl Hecht, Sondheim vor der Rhön, Germany) for 1 min. Subsequently, 0.1 mL of the reconstituted suspension was inoculated onto a culture plate of Sabouraud dextrose agar (Oxoid, Basingstoke, England) and incubated aerobically at 37°C for 48 h. *Candida* colonies were identified based on the colony color, texture and morphology, and the purity of the culture was determined microscopically, using the wet mount technique with a ×100 objective. The colonies on the plate were counted, and the number was multiplied by 10 to calculate the number of colony-forming units per 1 mL of the rinse (CFU/mL). *Candida albicans* and other species were identified using the germ tube test[16] and the VITEK® 2 system with new colorimetric yeast cards (bioMérieux, Durham, USA).[17]

The study protocol was reviewed and approved by the Institutional Review Board at KAUH (ref No. 1/116/2018) in compliance with the Declaration of Helsinki.

Statistical analysis

The data was analyzed using the SPSS Statistics for Windows software, v. 17.0 (SPSS Inc., Chicago, USA). The Shapiro–Wilk test was used to check for the normality of continuous data. The χ^2 test was used for categorical data. The Mann–Whitney *U* test was used for non-normally distributed data. The level of significance was established at $p \leq 0.05$.

Results

Biographic and clinical data

This study included a total of 100 subjects. Each of the study groups was composed of 27 (54%) males and 23 (46%) females. The mean age in the HLS and HLNS groups was 55.6 ±10.5 years and 55.1 ±10.8 years, respectively, and the age range was 37–80 years.

At the time of the study, 28 (56%) subjects in the HLS group were on simvastatin, 14 (28%) were on atorvastatin, 5 (10%) were on fluvastatin, and 3 (6%) were on lovastatin. At the time of inclusion in the study, 42 (84%) of the HLS subjects were on a 20 mg daily dose, while only 3 (6%) were on 10 mg, 3 (6%) were on 40 mg, 1 (2%) was on 60 mg, and 1 (2%) was on 80 mg.

Oral *Candida* carriage

As presented in Table 1, the prevalence and mean count of the isolated oral *Candida* species were significantly lower in the HLS group as compared to the HLNS group. *Candida albicans* was the most commonly isolated species in both groups. In addition, a lower number of species was isolated in the HLS group ($n = 4$) as compared to the HLNS group ($n = 6$). The prevalence of *C. albicans* was lower, although not statistically significantly, among the HLS group (18%) as compared to the HLNS group (30%).

The statistical analysis showed no significant association between either the prevalence or the mean count of the isolated oral *Candida* species and the subject's age or the duration of statin therapy (Table 2).

Discussion

To the author's knowledge, this pioneer clinical study is the first to investigate the effect of statin therapy on the oral carriage of *Candida* species. The finding that the oral cavity of 60% of the hyperlipidemic patients who were not on statin therapy was colonized by *Candida* is comparable to the 57.7% prevalence previously reported among the general population from the same geographic area,[18] which gives validity to the current results. It may be suggested that hyperlipidemia per se is not a factor that affects oral *Candida* carriage. *Candida albicans* has previously been shown to be the most common *Candida* species isolated from the oral cavity,[3] which is consistent with the results of the present study. The concentrated oral rinse technique used in this study is known for its superiority, both qualitatively and quantitatively, in the overall candidal sampling of the oral cavity.[15] In an attempt to neutralize the possible effects of any confounders, the study group and control group subjects were closely matched for gender, age and the dental status, and strict inclusion and exclusion criteria were implemented.

Table 1. Prevalence and mean count of different *Candida* species isolated from the study subjects

Variable	HLS ($n = 50$)	HLNS ($n = 50$)	*p*-value	Total ($N = 100$)
C. albicans	9 (18)	15 (30)	0.160	24 (24)
C. kefyr	1 (2)	3 (6)	0.617	4 (4)
C. dubliniensis	7 (14)	3 (6)	0.318	10 (10)
C. spherica	0 (0)	5 (10)	0.056	5 (5)
C. krusei	0 (0)	1 (2)	1.000	1 (1)
C. glabrata	3 (6)	3 (6)	1.000	6 (6)
Total	20 (40)	30 (60)	0.040*	50 (50)
Count [CFU/mL]	49.1 ±18.0	162.6 ±28.0	0.0001*	105.8 ±24.0

Data presented as number (percentage) (*n* (%)) or as mean ± standard deviation (*M* ±*SD*). HLS – hyperlipidemia statin group; HLNS – hyperlipidemia non-statin group; CFU – colony-forming unit; * statistically significant.

Table 2. Relationship between the prevalence and mean count of the isolated oral *Candida* species and the patient's age and the duration of statin therapy in the HLS group

Variable		Prevalence	*p*-value	Count [CFU/mL]	*p*-value
Age [years]	<50 (*n* = 18)	8 (44.4)	1.000	36.9 ±11.0	0.950
	51–60 (*n* = 16)	6 (37.5)		26.9 ±5.0	
	>60 (*n* = 16)	6 (37.5)		83.7 ±18.0	
	total (*n* = 50)	20 (40.0)		49.1 ±18.0	
Duration of statin therapy [months]	<24 (*n* = 20)	9 (45.0)	1.000	18.2 ±6.0	0.860
	25–70 (*n* = 19)	7 (36.8)		86.4 ±17.0	
	>70 (*n* = 11)	4 (36.4)		27.3 ±12.0	

Data presented as *n* (%) or as *M* ±*SD*.

The antifungal potential of statins has been described in several animal and laboratory studies.[6–10,19] To date, this study is the first clinical investigation to demonstrate that statin therapy is associated with a significant reduction in oral *Candida* colonization, both qualitatively and quantitatively. The antifungal mechanisms of statins are not clear. Statins lower cholesterol levels in blood through their action on HMG-CoA reductase, an essential enzyme for the biosynthesis of cholesterol. It is widely believed that fungal HMG-CoA reductases may also be inhibited by statins, resulting in reduced levels of cell wall ergosterol (the fungal equivalent of cholesterol), and the subsequent disruption of the synthesis of the candidal cell wall, which leads to the loss of intracellular components and cell death.[5] This hypothesis was indirectly supported by the research which determined that the addition of exogenous ergosterol to the statin–*Candida* assay overcame the inhibitory effect of simvastatin.[19,20] Ting et al. in their systematic review described other possible antifungal mechanisms of statins.[21] Statins may indirectly affect fungal cell signaling, proliferation and differentiation through the inhibition of the synthesis of isoprenoid. In addition, statins can adversely affect the synthesis of HMG-CoA reductase-associated fungal products, such as farnesol, which is a virulence factor of *C. albicans*. Moreover, statins may also cause the direct apoptosis of fungal cells by inhibiting protein isoprenylation. Statins inhibit the synthesis of different prenyl groups in fungi, which are important for the attachment of lipids to the heterotrimeric G-proteins gamma subunit, guanosine triphosphate(GTP)-binding protein (Ras) and Ras-like proteins. Statins may also inhibit G-protein actions and Ras or Ras-like signaling, which are vital for fungal proliferation and differentiation.[21] In summary, it is likely that more than a single mechanism may be acting simultaneously to inhibit fungal activity.

According to the current results, the duration of statin therapy did not seem to have a significant effect on oral *Candida* carriage. It happened that the majority of the study subjects (84%) were on a 20 mg daily dose and the other 16% were administered other doses, which rendered any statistical attempt to explore the relationship between *Candida* colonization and the statin dose inappropriate. This unavoidable shortcoming was a stumbling block in the way of studying the effect of different statin types and doses on oral *Candida* colonization, which could be overcome in future studies. Nevertheless, laboratory studies have shown simvastatin and atorvastatin to have a superior inhibitory effect on *C. albicans* over other statins.[6,10,16] At the time of the present study, 84% of the HLS group were on either simvastatin or atorvastatin.

While reviewing the literature, it was observed that not all *Candida* species were equally susceptible to the statin inhibitory effect, but *C. albicans* was the most sensitive.[22] This may explain the wide discrepancy in the prevalence of *C. albicans* between the HLS group (18%) and the HLNS group (30%) observed in the present study. It would be valuable for this observation to undergo further investigation.

Whether the achievable serum levels of therapeutic doses of statins are inhibitory to fungal activity is still a matter of debate. Several studies have shown that high concentrations of statins, well above the maximum achievable serum level in humans in therapeutic doses, were needed to induce an antifungal effect.[5,19] On the other hand, a study by Nogueira Brilhante et al. demonstrated that the minimum inhibitory concentration (MIC) of simvastatin against *C. albicans* was similar to the serum levels of the drug when administered to control blood cholesterol.[6] It is tempting to speculate about the presence of genotyping differences in susceptibility to statins among *Candida* species. Further clinical studies are needed to investigate whether the therapeutic doses of statins can produce salivary levels of the drug that are sufficient to inhibit fungal activity, and to elucidate whether statins combined with antifungal agents can aid in controlling recalcitrant oral candidiasis. Research has shown that when statins are combined with a number of clinically used antifungal agents, they act synergistically and result in substantial decreases in the the therapeutic doses of antifungal agents.[5,6,10] According to these results, whether statins can be applied in the current clinical practice to treat oral fungal infections needs more investigation.

Limitations

There are limitations to this study. The sample size was relatively small (50 statin users and 50 non-users). Due to the strict selection criteria that were adopted, it was difficult to recruit more subjects within the study time frame. It is possible that future larger-scale studies will help elucidate the effect of the statin dose or the duration of therapy on oral fungi, which was not possible in this study. The largest proportion of patients (84%) was on a 20 mg daily statin dose, which rendered exploring the relationship between oral *Candida* colonization and different statin doses not feasible.

Conclusions

Within the limitations of this study, the therapeutic dose of statins was associated with a reduction in the prevalence and count of oral *Candida*. Larger-scale studies are needed to define the relationship between this antifungal effect and the dose, type and duration of statin therapy, and its efficacy in controlling clinical oral candidal infection.

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Institutional Review Board at KAUH (ref. No. 1/116/2018) in compliance with the Declaration of Helsinki and all participants provided written informed consent prior to the investigation.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Azmi Darwazeh https://orcid.org/0000-0002-5802-1774
Hisham Al-Shorman https://orcid.org/0000-0002-1312-0090
Bayan Mrayan https://orcid.org/0000-0002-1736-5319

References

1. Abujbara M, Batieha A, Khader Y, Jaddou H, El-Khateeb M, Ajlouni K. The prevalence of dyslipidemia among Jordanians. *J Lipids*. 2018;2018:6298739. doi:10.1155/2018/6298739
2. Lippi G, Mattiuzzi C, Cervellin G. Statins popularity: A global picture. *Br J Clin Pharmacol*. 2019;85(7):1614–1615. doi:10.1111/bcp.13944
3. Bandara HM, Samaranayake LP. Viral, bacterial, and fungal infections of the oral mucosa: Types, incidence, predisposing factors, diagnostic algorithms, and management. *Periodontol 2000*. 2019;80(1):148–176. doi:10.1111/prd.12273
4. Athyros VG, Tziomalos K, Karagiannis A, Mikhailidis DP. Statins and cardiovascular events in patients with end-stage renal disease on hemodialysis. The AURORA results suggest the need for earlier intervention. *Curr Vasc Pharmacol*. 2009;7(3):264–266. doi:10.2174/157016109788340712
5. Nyilasi I, Kocsubé S, Krizsán K, et al. Susceptibility of clinically important dermatophytes against statins and different statin-antifungal combinations. *Med Mycol*. 2014;52(2):140–148. doi:10.3109/13693786.2013.828160
6. Noguiera Brilhante RS, Caetano EP, de Oliveira JS, et al. Simvastatin inhibits planktonic cells and biofilms of *Candida* and *Cryptococcus* species. *Braz J Infect Dis*. 2015;19(5):459–465. doi:10.1016/j.bjid.2015.06.001
7. Nyilasi I, Kocsubé S, Krizsán K, et al. *In vitro* synergistic interactions of the effects of various statins and azoles against some clinically important fungi. *FEMS Microbiol Lett*. 2010;307(2):175–184. doi:10.1111/j.1574-6968.2010.01972.x
8. Wikhe K, Westermeyer C, Macreadie IG. Biological consequences of statins in *Candida* species and possible implications for human health. *Biochem Soc Trans*. 2007;35(Pt 6):1529–1532. doi:10.1042/BST0351529
9. Ajdidi A, Sheehan G, Abu Elteen K, Kavanagh K. Assessment of the in vitro and in vivo activity of atorvastatin against *Candida albicans*. *J Med Microbiol*. 2019;68(10):1497–1506. doi:10.1099/jmm.0.001065
10. Lima WG, Alves-Nascimento LA, Andrade JT, et al. Are the statins promising antifungal agents against invasive candidiasis? *Biomed Pharmacother*. 2019;111:270–281. doi:10.1016/j.biopha.2018.12.076
11. Cuervo G, Garcia-Vidal C, Nucci M, et al. Effect of statin use on outcomes of adults with candidemia. *PLoS One*. 2013;8(10):e77317. doi:10.1371/journal.pone.0077317
12. Spanakis EK, Kourkoumpetis TK, Livanis G, Peleg AY, Mylonakis E. Statin therapy and decreased incidence of positive *Candida* cultures among patients with type 2 diabetes mellitus undergoing gastrointestinal surgery. *Mayo Clin Proc*. 2010;85(12):1073–1079. doi:10.4065/mcp.2010.0447
13. Rahal EA, Constantin WN, Zeidan N, Abdelnoor AM. Atorvastatin reduces the survival of *Candida albicans*-infected BALB/c mice. *Front Microbiol*. 2015;6:1474. doi:10.3389/fmicb.2015.01474
14. Welch ML, Liappis AP, Kan VL. Candidemia outcomes not improved with statin use. *Med Mycol*. 2013;51(2):219–222. doi:10.3109/13693786.2012.692490
15. Samaranayake LP, MacFarlane TW, Lamey PJ, Ferguson MM. A comparison of oral rinse and imprint sampling techniques for the detection of yeast, coliform and *Staphylococcus aureus* carriage in the oral cavity. *J Oral Pathol*. 1986;15(7):386–388. doi:10.1111/j.1600-0714.1986.tb00646.x
16. Mackenzie DW. Serum tube identification of *Candida albicans*. *J Clin Pathol*. 1962;15(6):563–565. doi:10.1136/jcp.15.6.563
17. Graf B, Adam T, Zill E, Göbel UB. Evaluation of the VITEK 2 system for rapid identification of yeasts and yeast-like organisms. *J Clin Microbiol*. 2000;38(5):1782–1785. doi:10.1128/JCM.38.5.1782-1785.2000
18. Darwazeh AM, Hammad MM, Al-Jamaei AA. The relationship between oral hygiene and oral colonization with *Candida* species in healthy adult subjects. *Int J Dent Hyg*. 2010;8(2):128–133. doi:10.1111/j.1601-5037.2009.00407.x
19. Liu G, Vellucci VF, Kyc S, Hostetter MK. Simvastatin inhibits *Candida albicans* biofilm in vitro. *Pediatr Res*. 2009;66(6):600–604. doi:10.1203/PDR.0b013e3181bd5bf8
20. Macreadie IG, Johnson G, Schlosser T, Macreadie PI. Growth inhibition of *Candida species* and *Aspergillus fumigatus* by statins. *FEMS Microbiol Lett*. 2006;262(1):9–13. doi:10.1111/j.1574-6968.2006.00370.x
21. Ting M, Whitaker EJ, Albandar JM. Systematic review of the in vitro effects of statins on oral and perioral microorganisms. *Eur J Oral Sci*. 2016;124(1):4–10. doi:10.1111/eos.12239
22. Szenzenstein J, Gácser A, Grózer Z, et al. Differential sensitivity of the species of *Candida parapsilosis* sensu lato complex against statins. *Mycopathologia*. 2013;176(3):211–217. doi:10.1007/s11046-013-9689-1

Comparative evaluation of the antimicrobial effects of probiotic milk and probiotic powder on the salivary *Streptococcus mutans* counts and the plaque scores in children aged 3–6 years: A randomized controlled trial

Palak Janiani[A–D], Vignesh Ravindran[A,E,F]

Department of Pediatric and Preventive Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):99–104

Address for correspondence
Palak Janiani
E-mail: palakjaniani@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on May 15, 2021
Reviewed on June 18, 2021
Accepted on July 2, 2021

Published online on March 17, 2022

Abstract

Background. Probiotics have been shown to have a positive influence on systemic and oral health. The prevention of dental caries and gingival diseases through the consumption of probiotics has been studied extensively.

Objectives. The aim of this research was to determine the effects of a short-term intake of probiotic milk and powder on the salivary levels of *Streptococcus mutans* (*S. mutans*) and the plaque scores in children.

Material and methods. In this short-term study, 34 healthy 3–6-year-old children were randomly assigned to group A (control), group B (enKor-D) or group C (Yakult). The probiotics were taken for 7 days. To screen for the amount of *S. mutans* measured in colony-forming units per milliliter of saliva (CFU/mL), unstimulated saliva samples were collected and cultured on Mitis Salivarius Agar plates before and after the intervention. The plaque scores were also recorded at pre- and post-intervention visits.

Results. A statistically significant reduction of salivary *S. mutans* was recorded after the consumption of probiotic milk (Yakult®) and powder (enKor®-D) ($p < 0.05$), with the decrease being greater for the enKor-D group. However, only the consumption of Yakult decreased the plaque scores significantly ($p < 0.05$).

Conclusions. A short-term use of Yakult and enKor-D can have a cariostatic effect by lowering oral microbial load in children with certain risk profiles. Further research is required to confirm this probiotic effect over a long-term period. Prior to prescribing or promoting Yakult or enKor-D as an adjunct caries prevention therapy for children, a thorough risk evaluation may be needed.

Keywords: probiotics, caries, dental plaque index, *Streptococcus mutans*

Cite as
Janiani P, Ravindran V. Comparative evaluation of the antimicrobial effects of probiotic milk and probiotic powder on the salivary *Streptococcus mutans* counts and the plaque scores in children aged 3–6 years: A randomized controlled trial. *Dent Med Probl.* 2022;59(1):99–104. doi:10.17219/dmp/139731

DOI
10.17219/dmp/139731

Introduction

Early childhood caries (ECC) is a distressingly widespread disease among children in both developed and developing countries, with *Streptococcus mutans* (*S. mutans*) considered to be the chief causative organism.[1] Despite the use of conventional caries treatment measures ranging from invasive approaches, such as extractions/restorations, to minimally invasive or non-invasive methods, such as the use of fluorides and remineralizing agents, the prevalence of ECC does not display a substantial decline.[2] This can be attributed to most individuals neglecting oral health care, either due to the high cost of treatment or because of the lack of awareness about simple preventive methods that can be incorporated in one's daily life. One of such upcoming preventive tools against the development of caries is the use of probiotics.

The concept of probiotics, which was introduced by Élie Metchnikoff,[3] dates back to 1908. Originating from the Greek word meaning "for life", probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.[4] Their effect on gut flora has been widely studied and it is believed that they have a similar effect in the oral cavity; they prohibit *S. mutans* from colonizing tooth surfaces, preventing the development of dental plaque and, as a result, decreasing the risk of dental caries.

A household name in the field of probiotics for over 50 years has been Yakult®, a well-known probiotic product originating from Japan and Taiwan. Its constituents include artificially sweetened skimmed milk with a suspension of *Lactobacillus casei* strain Shirota (LcS). Studies have shown that Yakult can be consumed on a daily basis by all children for up to 55 months without the fear of developing adverse effects.[5] Taking into account the high acceptability, minimal side effects and enhanced flavor of Yakult, it would be of great importance to determine the probiotic effects of LcS as a caries-preventing factor in people consuming this dairy beverage.

A newly emerging probiotic, enKor®-D, is an India-based product in the form of a sachet containing a blend of 2.7 billion colony-forming units (CFU) of different species of *Lactobacillus* bacteria. It is claimed that along with reducing the prevalence of caries, it can also alleviate gingivitis, periodontitis, halitosis, and a sore throat.[6] As opposed to Yakult and other probiotics, enKor-D is in a powdered form; it is to be consumed by sprinkling the contents of the sachet onto the tongue and allowing the powder to dissolve. Having simple administration instructions for all age groups, this probiotic could be the "next Yakult" in the industry.

Although the correlation between probiotics and oral health is not a satisfactorily ventured avenue, it has been suggested that consuming these products from an early age promotes the development of healthy bacterial strains in our bodies. The need to identify a suitable administration vehicle for these probiotic species is the first step. Currently, the globally fancied modes include milk, yogurt, ice cream, and cheese, but the ideal vehicle which could be widely accepted across age groups has not been confirmed yet. This study was conducted to determine the immediate short-term effects of probiotic milk and the newly emerging probiotic powder in a 3–6-year-old age group with the aim of comparing the salivary levels of *S. mutans* and the plaque scores before and after the administration of the probiotics.

Material and methods

This parallel, randomized controlled trial (RCT) was carried out after obtaining approval from the institutional review board (IHEC/SDC/PEDO-2001/21/24) at a dental university setting (Saveetha Dental College and Hospitals, Chennai, India). It was conducted over a 1-month period, from December 2020 to January 2021. Children aged 3–6 years who reported to the Department of Pediatric and Preventive Dentistry at Saveetha Dental College and Hospitals and had a deft (for primary teeth – decayed, extracted, filled teeth)/DMFT (for permanent teeth – decayed, missing, filled teeth) score <2 were considered to be eligible for the study. Children having a history of dental treatment in the past 6 months, including topical fluoride application, severe medical conditions, any allergy to dairy products, and those on any medications were excluded from the study.

A sample size of 27 was determined with an 80% power of study at a significance level of 0.05 to detect a discrepancy between the groups. To account for a potential loss to follow-up of up to 20%, 7 children were added, bringing the overall number of participants to 34. The 34 children were randomly allocated with a coin toss to the control group or either test group (another coin toss) after their dental status was checked with a mouth mirror and a probe under operative light in a conventional dental chair. Allocation was performed by one of the investigators (VR). The allocation process is depicted in a flow chart (Fig. 1).

The study was explained to the parents before data was collected, and their informed consent was received. The included subjects were randomly divided into 3 groups: group A (control); group B (enKor-D); and group C (Yakult). The single-blinding procedure was followed, in which the individuals examining the subjects and gathering data were unaware of the group the participant belonged to. Subjects in group A were not given anything to consume, whereas those in group B received 1 sachet of probiotic powder (enKor-D; Tenshi LifeCare, Bengaluru, India) and those in group C received 10 mL of probiotic milk (Yakult; Yakult Danone India, New Delhi, India) for 7 days. The parents were instructed about the mode

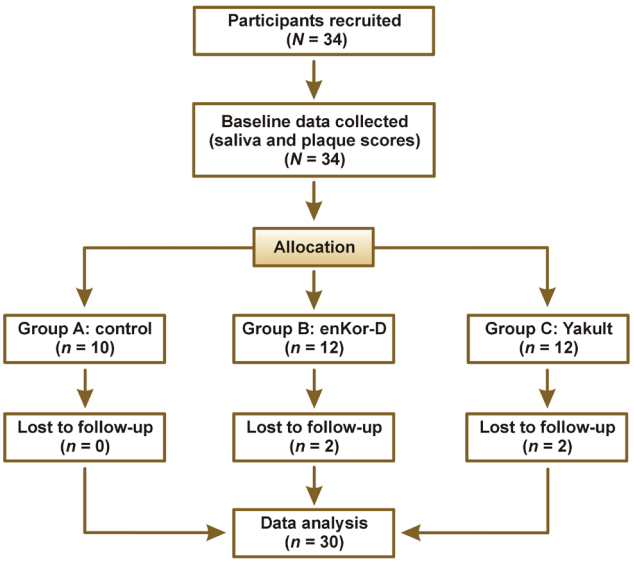

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram

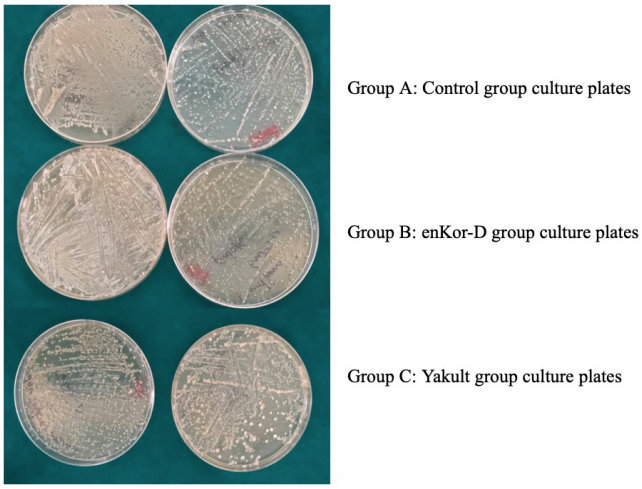

Fig. 2. Salivary samples inoculated on Mitis Salivarius Agar culture plates
Intervention – the consumption of the probiotic;
left side – pre-intervention; right side – post-intervention (in group A, no intervention was employed).

of administration of enKor-D, which is to be consumed by sprinkling the contents of the sachet onto the tongue and allowing the powder to dissolve. Rather than drinking Yakult directly, the subjects from group C were given similar orders, which included keeping the drink in their mouth for 1 min, and then swallowing it. This was done to ensure the topical effect of Yakult, similar to that of enKor-D. The parents were informed that their children should avoid rinsing their mouths and abstain from any liquid or solid intake for a period of 30 min after having the probiotic. They were asked to send the investigator a video of their child taking the probiotic every day. Common dietary recommendations and oral hygiene instructions were to be followed by all groups during the study period.

At the 1st visit, the collection of baseline unstimulated saliva into a 20-milliliter disposable sterile container was carried out. The salivary samples were immediately taken to the laboratory for culturing. These samples were inoculated for 48 h at 37°C on freshly prepared Mitis Salivarius Agar culture plates (Mitis Salivarius Agar Base; HiMedia Laboratories, Mumbai, India) and subjected to microbial analysis (Fig. 2). An automated colony counter (LT-37; Labtronics, Panchkula, India) was used to determine the amount of *S. mutans*, which was measured in CFU/mL of saliva. The Silness–Löe plaque index (PI) was used to record the plaque scores for all subjects. These findings were tabulated and used to establish baseline statistics.

After a 7-day period of probiotic consumption (i.e., on day 8), unstimulated saliva was collected again into a different sterile container and incubated, after which the colonies were counted. The PI scores were also recorded. The data was tabulated in the same Microsoft Office Excel spreadsheet (v. 2010; Microsoft, Redmond, USA) as the baseline data.

Statistical analysis

The data was exported and subjected to statistical analysis with the use of the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA).

Descriptive statistics were employed with regard to the plaque scores and the *S. mutans* counts. Parametric tests were used to determine statistically significant differences in both the microbial counts and the plaque scores. The intergroup comparisons (between the 3 groups) of the mean PI scores and *S. mutans* counts at both time points (before and after) were performed using the analysis of variance (ANOVA). Tukey's post hoc honestly significant difference (HSD) test was carried out for multiple comparisons. The intragroup comparisons of the mean PI scores and *S. mutans* counts in all 3 groups were made using the paired *t* test. For all analyses, $p < 0.05$ was considered statistically significant.

Results

Thirty-four children aged 3–6 years were recruited for this study. Four children dropped out after the 1st appointment; 2 were from the enKor-D group and 2 were from the Yakult group.

According to the findings of this research, all groups showed a decrease in the PI scores after 7 days as compared to baseline, but only the participants who consumed Yakult showed a statistically significant reduction ($p = 0.016$) (Table 1). The intergroup comparisons of the PI scores revealed no statistically significant differences between the groups (Table 1). A reduction in the *S. mutans* counts was also observed in all groups, with the control group showing the smallest decline (Table 2). This reduction was statistically significant after the consumption

of both probiotics, with enKor-D providing a more pronounced decrease from 560.3 CFU/mL to 340.1 CFU/mL (Table 2).

These comparisons indicate that during the study period, both probiotics decreased the salivary *S. mutans* levels, but only the consumption of Yakult decreased the plaque scores significantly ($p < 0.05$). No side effects were reported in any of the groups.

Discussion

Early childhood caries is a common disease globally, showing a varied prevalence, with less developed countries reporting an incidence of up to 70%.[7] Not only does ECC have a long-term negative effect on dentition, but it may also have an effect on a child's general health, cognitive abilities and overall quality of life; dental caries pain has a detrimental effect on a child's physical well-being, sleep habits, and desire to understand and practice daily activities.[7] These findings highlight the urgency of researching novel self-administered prevention approaches to supplement the current evidence-based guidelines for ECC regulation. As a result, the use of a number of antiplaque products has been attempted to enhance oral hygiene, including local and systemic fluorides, pit and fissure sealants, and newer options, including xylitol, triclosan and probiotics.[8]

Bacterial therapy, also known as a replacement therapy, is a form of treatment that uses harmless bacteria rather than pathogenic microorganisms to cure diseases. Due to their beneficial effects in treating caries, gingivitis and halitosis, probiotics have been added to toothpastes and mouthwashes. They present no chronic toxicity and have beneficial systemic effects when ingested. As a result, probiotics are truly novel, cutting-edge agents that can be used to treat dental caries, including ECC, with minimal side effects.[9] According to Hedayati-Hajikand et al., a long-term use of dairy products containing probiotic strains may aid in the prevention and treatment of childhood caries.[10]

Poureslami et al. studied the effects of a dairy product of a probiotic nature (Espar) on the salivary calcium and *S. mutans* levels.[11] They concluded that increasing the calcium content of dental plaque, which can be successfully achieved by eating dairy products, can prevent enamel demineralization.[11] In the present study, milk was selected for analysis in one test group, since it possesses anticariogenic properties due to the casein, calcium and phosphorus content. Above all, milk is popular and commonly liked. The ideal vehicle for the delivery of probiotics has not been established yet; enKor-D, being a new probiotic, was included in this study, as it uses a new delivery system for administering probiotics.

Two of the most commonly used probiotic bacterial genera are *Bifidobacterium* and *Lactobacillus*.[12] The *Lactobacillus* species have been found to prevent the development of the *S. mutans* colonies when the probiotics are applied during oral biofilm creation.[13] The significant drop in the salivary levels of *S. mutans* found in this study may be explained by the potential mechanisms of action of probiotics, which include the inhibition of bacterial adhesion, preventing the colonization of the oral cavity early on, interspecies association, and immunomodulation.[14]

Table 1. Comparison of the plaque index (PI) scores within and between the study groups

Time point	Group A (control)	Group B (enKor-D)	Group C (Yakult)	Intergroup comparison *p*-value (ANOVA)
Baseline/Pre-intervention	0.53 ±0.15	0.47 ±0.19	0.60 ±0.16	0.243
Post-intervention	0.44 ±0.15	0.37 ±0.21	0.41 ±0.17	0.678
Intragroup comparison *p*-value (paired *t* test)	0.089	0.097	0.016*	–

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

Table 2. Comparison of the *Streptococcus mutans* (*S. mutans*) counts [CFU/mL] within and between the study groups

Time point	Group A (control)	Group B (enKor-D)	Group C (Yakult)	Intergroup comparison *p*-value (ANOVA)
Baseline/Pre-intervention	468.1 ±169.3	560.3 ±121.6	566.5 ±109.6	0.212
Post-intervention	452.7 ±172.4	340.1 ±111.4	395.4 ±112.0	0.195
Intragroup comparison *p*-value (paired *t* test)	0.092	0.003*	0.005*	–

Data presented as *M ±SD*. * statistically significant.

As opposed to this study, previous studies showed that probiotics containing *Lactobacillus reuteri* (*L. reuteri*) led to a substantial decrease in the plaque scores due to their anti-inflammatory action.[15–17] The current findings show that a significant decrease in the plaque scores was observed only with the consumption of Yakult, which does not contain that particular species of *Lactobacillus*. On the other hand, the use of enKor-D did not result in a significant decrease in the plaque scores, even though it contains *L. reuteri*. The different tooth brushing habits of children may have played a role in lowering the plaque scores in the Yakult group more than in the enKor-D group.

As shown in Table 2, the salivary levels of *S. mutans* recorded after 7 days of probiotic consumption were statistically significantly lower as compared to baseline ($p < 0.05$). The findings of this research match those of Chinnappa et al., who observed differences after 1 h, but they became statistically significant after 7 days.[18] Our analysis showed a statistically significant decrease in salivary *S. mutans* after the intake of probiotic milk and powder, which is consistent with previous research by Jindal et al.[19] Chuang et al. presented results that were contrasting to ours; they showed no statistically significant differences between the control and probiotic groups.[20]

The beneficial effects of probiotics are transient and they do not persist for long after the discontinuation of consumption.[21] Depending on the vehicle utilized for the probiotic, different studies suggest varying washout times. In a study by Mahantesha et al., the participants consuming probiotic ice cream (Amul) showed reduced *S. mutans* levels when compared to a probiotic drink (Yakult) after a 90-day washout period.[22] The participants in a study by Manoharan et al. were given homemade probiotic curd to consume and the gradual recolonization of the studied organisms was observed after a washout period of 14 days.[23] Hence, further studies would be required to assess the long-term efficacy of probiotics, based on their washout periods.

Limitations

Even though only short-term probiotic administration was evaluated in this study, a substantial decrease in the *S. mutans* cariogenic bacterial count was noticed. It is possible that taking probiotics for a longer period of time would help deter caries from developing. Long-term research on the effects of probiotics in reducing bacterial counts would be beneficial. Another limitation of this study is a relatively small sample size, suggesting that the study could be underpowered. On the other hand, the major effects observed in the respondent sample suggest that the possible impact merits further study.

Conclusions

Probiotic milk (Yakult) was found to be as effective as probiotic powder (enKor-D) in decreasing the salivary *S. mutans* levels. Yakult was more effective in reducing the plaque scores over a 7-day period. Since they are ingestible and have limited systemic toxic effects, probiotics can be used as an alternative to other preventive measures for dental caries, especially in children above the age of 3 years. The use of such agents for an extended period of time, however, should be analyzed in further studies.

Ethics approval and consent to participate

This study was carried out after obtaining approval from the institutional review board (IHEC/SDC/PEDO-2001/21/24) at Saveetha Dental College and Hospitals, Chennai, India. The participants' parents provided written informed consent prior to the investigation.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Palak Janiani https://orcid.org/0000-0002-4957-3083
Vignesh Ravindran https://orcid.org/0000-0002-7534-3636

References

1. Marcenes W, Kassebaum NJ, Bernabé E, et al. Global burden of oral conditions in 1990–2010: A systematic analysis. *J Dent Res*. 2013;92(7):592–597. doi:10.1177/0022034513490168
2. Ashwin D, Ke V, Taranath M, Ramagoni NK, Nara A, Sarpangala M. Effect of probiotic containing ice-cream on salivary *Mutans Streptococci* (SMS) levels in children of 6–12 years of age: A randomized controlled double blind study with six-months follow up. *J Clin Diagn Res*. 2015;9(2):ZC06–ZC09. doi:10.7860/JCDR/2015/10942.5532
3. Mackowiak PA. Recycling Metchnikoff: Probiotics, the intestinal microbiome and the quest for long life. *Front Public Health*. 2013;1:52. doi:10.3389/fpubh.2013.00052
4. FAO Food and Nutrition Paper 85. Probiotics in food: Health and nutritional properties and guidelines for evaluation. Rome, Italy: World Health Organization, Food and Agriculture Organization of the United Nations; 2006:3. http://www.fao.org/3/a0512e/a0512e.pdf. Accessed March 21, 2021.
5. Lin YTJ, Chou CC, Hsu CYS. Effects of *Lactobacillus casei* Shirota intake on caries risk in children. *J Dent Sci*. 2017;12(2):179–184. doi:10.1016/j.jds.2016.09.005
6. Velbiom probiotics. https://velbiom.com/enKor-D-landing-page/. Accessed March 21, 2021.
7. Anil S, Anand PS. Early childhood caries: Prevalence, risk factors, and prevention. *Front Pediatr*. 2017;5:157. doi:10.3389/fped.2017.00157
8. Subramaniam P, Nandan N. Effect of xylitol, sodium fluoride and triclosan containing mouth rinse on *Streptococcus mutans*. *Contemp Clin Dent*. 2011;2(4):287–290. doi:10.4103/0976-237X.91790

9. Patil RU, Dastoor PP, Unde MP. Comparative evaluation of antimicrobial effectiveness of probiotic milk and fluoride mouthrinse on salivary *Streptococcus mutans* counts and plaque scores in children – an in vivo experimental study. *J Indian Soc Pedod Prev Dent*. 2019;37(4):378–382. doi:10.4103/JISPPD.JISPPD_45_19
10. Hedayati-Hajikand T, Lundberg U, Eldh C, Twetman S. Effect of probiotic chewing tablets on early childhood caries – a randomized controlled trial. *BMC Oral Health*. 2015;15(1):112. doi:10.1186/s12903-015-0096-5
11. Poureslami H, Pishbin L, Eslaminejad Z, Moqadam FJ, Farokhi MR. The effects of a dairy probiotic product, espar, on salivary calcium and *mutans streptococci*. *J Dent Res Dent Clin Dent Prospects*. 2013;7(3):147–151. doi:10.5681/joddd.2013.023
12. Sidhu GK, Mantha S, Murthi S, Sura H, Kadaru P, Jangra JK. Evaluation of *Lactobacillus* and *Streptococcus mutans* by addition of probiotics in the form of curd in the diet. *J Int Oral Health*. 2015;7(7):85–89. PMID:26229377. PMCID:PMC4513783.
13. Lee SH, Kim YJ. A comparative study of the effect of probiotics on cariogenic biofilm model for preventing dental caries. *Arch Microbiol*. 2014;196(8):601–609. doi:10.1007/s00203-014-0998-7
14. Guarner F, Khan AG, Garisch J, et al. World Gastroenterology Organisation Global Guidelines: Probiotics and prebiotics October 2011. *J Clin Gastroenterol*.2012;46(6):468–481.doi:10.1097/MCG.0b013e3182549092
15. Vicario M, Santos A, Violant D, Nart J, Giner L. Clinical changes in periodontal subjects with the probiotic *Lactobacillus reuteri* Prodentis: A preliminary randomized clinical trial. *Acta Odontol Scand*. 2013;71(3–4):813–819. doi:10.3109/00016357.2012.734404
16. Kumar V, Singhal R, Rastogi P, Lal N, Pandey S, Mahdi AA. Localized probiotic-guided pocket recolonization in the treatment of chronic periodontitis: A randomized controlled clinical trial. *J Periodontal Implant Sci*. 2021;51(3):199–212. doi:10.5051/jpis.2004140207
17. Vivekananda MR, Vandana KL, Bhat KG. Effect of the probiotic *Lactobacilli reuteri* (Prodentis) in the management of periodontal disease: A preliminary randomized clinical trial. *J Oral Microbiol*. 2010;2. doi:10.3402/jom.v2i0.5344
18. Chinnappa A, Konde H, Konde S, Raj S, Beena JP. Probiotics for future caries control: A short-term clinical study. *Indian J Dent Res*. 2013;24(5):547–549. doi:10.4103/0970-9290.123363
19. Jindal G, Pandey RK, Agarwal J, Singh M. A comparative evaluation of probiotics on salivary *mutans streptococci* counts in Indian children. *Eur Arch Paediatr Dent*. 2011;12(4):211–215. doi:10.1007/BF03262809
20. Chuang LC, Huang CS, Ou-Yang LW, Lin SY. Probiotic *Lactobacillus paracasei* effect on cariogenic bacterial flora. *Clin Oral Investig*. 2011;15(4):471–476. doi:10.1007/s00784-010-0423-9
21. Sutula J, Coulthwaite LA, Thomas LV, Verran J. The effect of a commercial probiotic drink containing *Lactobacillus casei* strain Shirota on oral health in healthy dentate people. *Microb Ecol Health Dis*. 2013;24. doi:10.3402/mehd.v24i0.21003
22. Mahantesha T, Reddy KM, Kumar NH, Nara A, Ashwin D, Buddiga V. Comparative study of probiotic ice cream and probiotic drink on salivary *Streptococcus mutans* levels in 6–12 years age group children. *J Int Oral Health*. 2015;7(9):47–50. PMID:26435616. PMCID:PMC4589718.
23. Manoharan V, Fareed N, Battur H, Praveena J, Ishwar P. Probiotic potential of daily consumption of homemade curd on dental plaque among schoolchildren: A randomized controlled trial. *J Indian Assoc Public Health Dent*. 2020;18(3):193–198. doi:10.4103/jiaphd.jiaphd_127_19

Distribution of biopsied gingival lesions according to the proceedings from the 2017 World Workshop classification: A three-year retrospective study

Vandana Gupta[1,A–D,F], Harpreet Kaur[2,A–D,F], Deepika Mishra[2,A–C,E,F], Vikender Singh Yadav[1,A–C,E,F], Sunny Kala[2,A–C,E,F]

[1] Division of Periodontics, Centre for Dental Education and Research, All India Institute of Medical Sciences, New Delhi, India
[2] Division of Oral Pathology and Microbiology, Centre for Dental Education and Research, All India Institute of Medical Sciences, New Delhi, India

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):105–110

Address for correspondence
Vikender Singh Yadav
E-mail: vikenderyadav@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on May 21, 2021
Reviewed on August 18, 2021
Accepted on August 24, 2021

Published online on March 29, 2022

Abstract

Background. The gingiva is a common site for neoplastic or non-neoplastic lesions. Neoplasms refer to progressive autonomous growth that can have either a benign or a malignant course. On the other hand, non-neoplastic lesions are mainly inflammatory, or occur as a reaction to some kind of irritation or low-grade injury.

Objectives. Assessing the frequency distribution of gingival lesions is important to optimize oral health care services. The present study retrospectively analyzed the frequency distribution of gingival lesions on the basis of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. The secondary objective was to compare this system with the 1999 International Workshop classification system.

Material and methods. The hematoxylin and eosin (H&E)-stained histopathological slides of the gingival lesions reported over the last 3 years (2018–2020) were retrieved from the archive of the Division of Oral Pathology and Microbiology at a tertiary care hospital in New Delhi, India. Correlating clinical, radiological and pathological details enabled the categorization of lesions according to the new classification system.

Results. In total, 73 gingival lesions were analyzed. Among these, reactive processes were the most frequent (39.73%), followed by inflammatory and immune conditions and lesions (26.03%), malignant tumors (21.92%), benign epithelial lesions (5.48%), and oral potentially malignant disorders (OPMDs) (5.48%). Genetic/developmental disorders were the least frequent (1.37%). However, as per the 1999 American Academy of Periodontology (AAP) system, the majority of lesions belonged to a non-specified category.

Conclusions. The frequency distribution of biopsied gingival lesions according to the 2017 World Workshop classification in comparison with the previous classification system showed that differences between the 2 systems could be attributed to heterogeneous terminology rather than to real geographical variations.

Keywords: distribution, inflammatory, frequency, non-neoplastic, benign epithelial

Cite as
Gupta V, Kaur H, Mishra D, Yadav VS, Kala S. Distribution of biopsied gingival lesions according to the proceedings from the 2017 World Workshop classification: A three-year retrospective study. *Dent Med Probl.* 2022;59(1):105–110. doi:10.17219/dmp/141555

DOI
10.17219/dmp/141555

Introduction

The gingiva is part of the oral mucosa that covers the alveolar processes of the jaws and surrounds the necks of the teeth. Human gingiva as well as other mucosal tissues may exhibit various non-plaque-induced pathological lesions, which can indicate systemic diseases or conditions.[1] These lesions are not caused by plaque and usually do not disappear after its removal. The severity of their clinical manifestation depends on the interaction with the underlying bacterial plaque.[2] Initially, the classification of non-plaque-induced gingival lesions, established by the American Academy of Periodontology (AAP), included gingival diseases of specific microbial origin, of genetic origin, manifestations of systemic conditions, traumatic lesions, foreign body reactions, and some lesions "not otherwise specified".[3] However, there are several non-neoplastic and neoplastic lesions that give rise to a unique set of lesions.[4] Neoplastic lesions can be benign or malignant, depending on their progressive autonomous growth. Non-neoplastic lesions may be inflammatory in origin, or can arise as a reaction to some kind of irritation or low-grade injury.[5] In the majority of cases, the clinical presentation of localized overgrowth is considered to be reactive and non-neoplastic in nature.[6] Hence an incisional or excisional biopsy for microscopic analysis is mandatory to prepare a definitive treatment plan. Periodontists, along with oral pathologists, make a collaborative team in terms of final accurate diagnosis, management and referral for treatment of gingival lesions.[7] Although a few studies in the literature have discussed the epidemiology of gingival lesions,[4,8,9] no study to date has reported the frequency distribution of these lesions according to the proceedings of the 2017 World Workshop on Periodontal and Peri-Implant Diseases and Conditions.[1]

The aim of the present study was to report the frequency distribution of the gingival lesions biopsied at a tertiary care referral center during the last 3 years as per the new classification scheme and to compare it with the 1999 International Workshop classification system.

Material and methods

This retrospective study was conducted at a tertiary care referral center at the Division of Oral Pathology and Microbiology, All India Institute of Medical Sciences, New Delhi, India. Ethical clearance was obtained from the institutional ethics committee (IEC-720/04.10.2019, RP-31/2019).

The histological slides of the gingival lesions biopsied in the years 2018–2020 were retrieved. The following criteria were used to analyze the records:

– inclusion criteria: clinically and histopathologically confirmed cases of gingival lesions irrespective of age and gender, where all demographic, clinical and other pertinent details were available;
– exclusion criteria: patients with reported systemic diseases, edentulous patients, those with drug-induced gingival enlargement, or those with missing clinical data.

The demographic details (age, gender), medical history (to determine the presence or absence of any systemic disease or long-term drug therapy) and dental records (history of any previous biopsy or radiographic findings, if any) of the patients who underwent a gingival biopsy were reviewed thoroughly by 2 independent examiners (DM, VSY). The histopathological examination was used as the gold standard to confirm the diagnosis in all cases. Fresh sections were taken from paraffin blocks, whenever required. The hematoxylin and eosin (H&E)-stained histopathological slides were evaluated by 3 pathologists (HK, DM, SK) to ascertain the final diagnosis, further categorizing it on the basis of the new classification of gingival lesions according to the 2017 World Workshop.[1]

Statistical analysis

The frequency distribution of the gingival lesions was determined using the Microsoft Office Excel spreadsheet (v. 2019; Microsoft, Redmond, USA).

Results

Among the 2,254 records retrieved from the archive of the Division of Oral Pathology and Microbiology, 73 records fulfilled the eligibility criteria and were included for further analysis (Fig. 1). The mean age of the patients was 39.1 years (age range: 7–69 years) (Fig. 2). The present study comprised 47 male patients and 26 female patients, with a male to female ratio of 1.8:1 (Fig. 3).

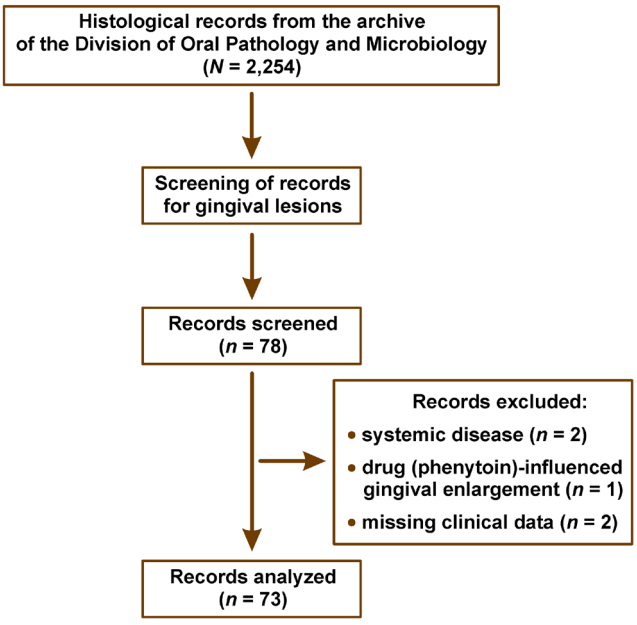

Fig. 1. Screening process for the identification of gingival lesions

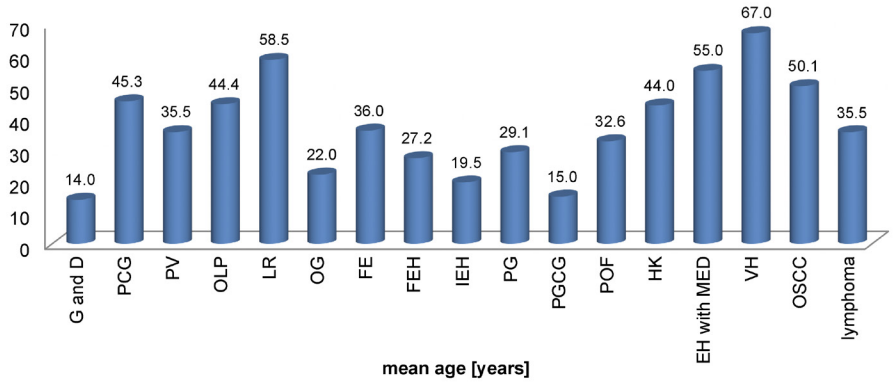

Fig. 2. Age distribution of the gingival lesions

G and D – genetic and developmental disorders; PCG – plasma cell gingivitis; PV – pemphigus vulgaris; OLP – oral lichen planus; LR – lichenoid reaction; OG – orofacial granulomatosis; FE – fibrous epulis; FEH – fibroepithelial hyperplasia; IEH – inflammatory epithelial hyperplasia; PG – pyogenic granuloma; PGCG – peripheral giant cell granuloma; POF – peripheral ossifying fibroma; HK – hyperkeratosis; EH with MED – epithelial hyperplasia with moderate epithelial dysplasia; VH – verrucous hyperplasia; OSCC – oral squamous cell carcinoma.

The most common histopathological categories, age and gender distribution, as well as comparisons between the 2 systems of classification (2018 vs. 1999) are summarized in Table 1.

As per the previous classification from 1999,[3] the majority of the non-plaque-induced gingival lesions were described under the "not otherwise specified" category (72.60%). However, according to the new classification system (Holmstrup et al., 2018),[1] the lesions were classified into 6 types (Fig. 4), with reactive processes being the most frequent (39.73%), followed by inflammatory and immune conditions and lesions (26.03%), malignant tumors (21.92%), benign epithelial lesions (5.48%), oral potentially malignant disorders (OPMDs) (5.48%), and genetic/developmental disorders (1.37%).

Reactive lesions were further categorized into 6 subtypes, with fibrous epulis and pyogenic granuloma most frequently reported. This was followed by fibroepithelial hyperplasia, peripheral ossifying fibroma, inflammatory epithelial hyperplasia, and peripheral giant cell granuloma. Inflammatory and immune conditions and lesions were divided into 3 subtypes, with a preponderance of plasma cell gingivitis under the category of hypersensitivity reactions, followed by lichen planus, lichenoid reactions and pemphigus vulgaris under the category of autoimmune diseases of skin and mucous membranes. Granulomatous inflammatory conditions were the least frequent. Among malignant tumors, oral squamous cell carcinoma (OSCC) showed a predominance, followed by lymphoma. Hyperkeratosis was reported as a benign epithelial lesion. Among OPMDs, epithelial hyperplasia with epithelial moderate dysplasia and verrucous hyperplasia were observed in gingival locations (Fig. 5).

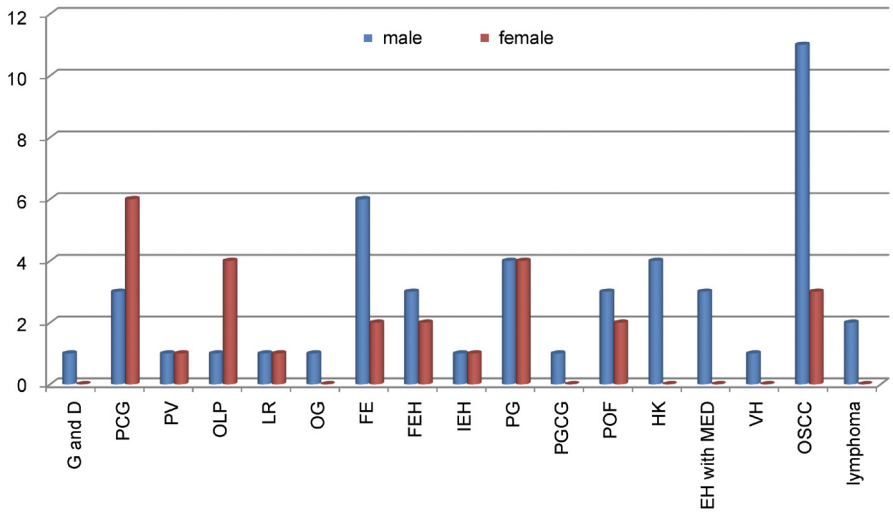

Fig. 3. Gender distribution of the gingival lesions

Table 1. Gingival lesions and conditions (working classification based on Holmstrup et al., 2018, compared with the American Academy of Periodontology (AAP) 1999 classification)

Classification	Category *n* (%)	Subcategory		Frequency distribution *n*	M:F ratio	Mean age [years]
Holmstrup et al., 2018	1. Genetic/developmental disorders 1 (1.37)	2 possibilities are considered in preferential order: (1) gingival fibromatosis; (2) atypical gingivitis		1	1:0	14.0
	2. Inflammatory and immune conditions and lesions 19 (26.03)	a. hypersensitivity reactions	plasma cell gingivitis	9	1:2	45.3
		b. autoimmune diseases of skin and mucous membranes	pemphigus vulgaris	2	1:1	35.5
			oral lichen planus	5	1:4	44.4
			lichenoid reaction	2	1:1	58.5
		c. granulomatous inflammatory conditions (orofacial granulomatosis)		1	1:0	22.0
	3. Reactive processes 29 (39.73)	fibrous epulis		8	3:1	36.0
		fibroepithelial hyperplasia		5	3:2	27.2
		inflammatory epithelial hyperplasia		2	1:1	19.5
		pyogenic granuloma		8	1:1	29.1
		peripheral giant cell granuloma		1	1:0	15.0
		peripheral ossifying fibroma		5	3:2	32.6
	4. Benign epithelial lesions 4 (5.48)	hyperkeratosis		4	4:0	44.0
	5. Oral potentially malignant disorders 4 (5.48)	epithelial hyperplasia with moderate epithelial dysplasia		3	3:0	55.0
		verrucous hyperplasia		1	1:0	67.0
	6. Malignant tumors 16 (21.92)	oral squamous cell carcinoma		14	11:3	50.1
		lymphoma		2	2:0	35.5
		total cases *n* (%)		73 (100)	1.8:1	39.1
AAP, 2019	specific bacterial, viral or fungal origin			–	–	–
	genetic origin 1 (1.37)			1	1:0	14.0
	gingival manifestation of a systemic condition 19 (26.03)			19	7:12	45.9
	traumatic lesion			–	–	–
	foreign body reaction			–	–	–
	not otherwise specified 53 (72.60)			53	2.8:1	36.6
	total cases			73	1.8:1	39.1

M – male; F – female.

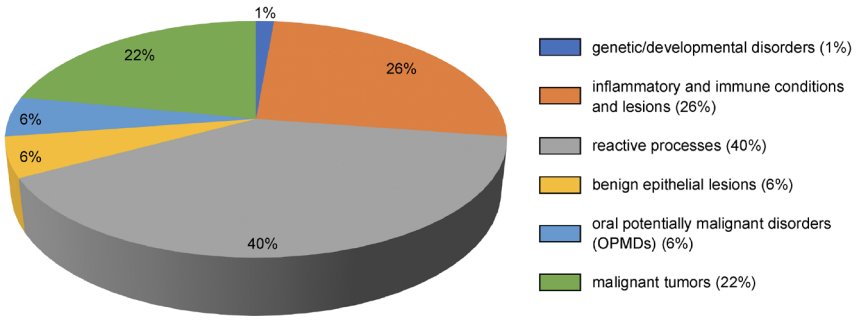

Fig. 4. Frequency distribution of the gingival lesions (the 2018 classification system)

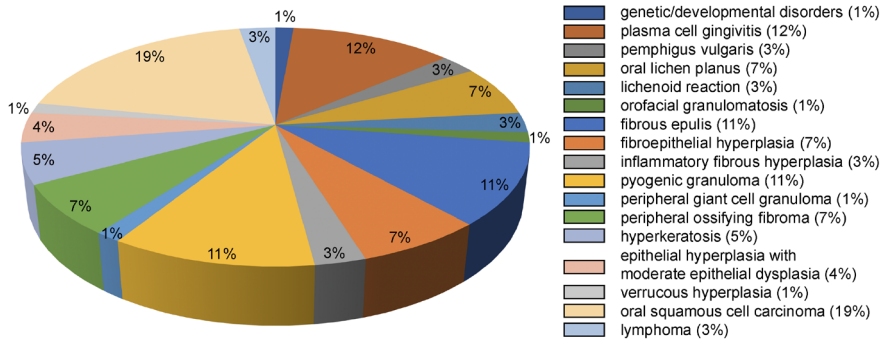

Fig. 5. Frequency distribution of the individual groups of gingival lesions (the 2018 classification system)

Discussion

To the best of our knowledge, the present study is the first to report biopsied gingival lesions based on the proceedings from the 2017 World Workshop presented by AAP and the European Federation of Periodontology (EFP).[1] Furthermore, a comparison with the AAP 1999 classification was an important hallmark of this study (Table 1). Non-plaque-induced gingival lesions were grouped into 6 categories. The most prevalent gingival lesions were reactive processes (39.73%), which is in accordance with a study by Kamath et al., who reported the prevalence to be 51%.[10] Among reactive processes, fibrous epulis and pyogenic granuloma were the most frequently reported lesions. The peak incidence of pyogenic granuloma was at 29.1 years, which is similar to the data reported in earlier studies.[11] In contrast to other studies in the literature, which reported a female predominance,[12] equal gender distribution was observed. Peripheral giant cell granuloma and peripheral ossifying fibroma were grouped together as reactive processes in this study, whereas previously they were reported under non-neoplastic lesions.[13,14] The current study suggests male predilection for peripheral giant cell granuloma and peripheral ossifying fibroma, which is consistent with the results provided by daSilva et al.[15]

The second most common non-plaque-induced gingival lesion category included inflammatory and immune conditions and lesions (26.03%), in which plasma cell gingivitis had the highest frequency in the age range of 14–82 years, with a female predominance. Recently, a similar study included plasma cell gingivitis under the category of inflammatory conditions as affecting primarily females with a mean age of 33.5 years.[16] In this study, lichen planus had the second highest frequency within autoimmune diseases of skin and mucous membranes, with a female predominance (M:F = 1:4). This is in agreement with some previous studies, where lichen planus was classified under non-neoplastic lesions affecting females significantly[14]; however, individual studies showed equal numbers for both genders, with a mean age of 47.5 years.[16]

Malignant tumors, including OSCC and lymphoma, represented the third most frequent non-plaque-induced gingival lesions (21.92%), with OSCC prevailing over lymphoma, which is similar to the previously reported findings.[4,17] Oral squamous cell carcinoma has been proven to be the most common type of cancer, reaching a frequency of 3.85% among all non-plaque-induced gingival lesions.[18] In addition, the gingiva is the third most common site for OSCC after carcinoma of the floor of the mouth and carcinoma of the tongue.[19,20] In the present study, its occurrence rate was higher in males as compared to females (M:F = 11:3) for those aged 35–65 years.

Oral potentially malignant disorders categorizing verrucous hyperplasia, followed by epithelial hyperplasia with moderate epithelial dysplasia, as well as benign epithelial lesions grouping hyperkeratosis accounted for 5.48% of all lesions, with a male predominance. These figures, along with the prevalence rate for epithelial lesions, were smaller as compared to those presented by Alblowi and Binmadi.[16] This might reflect geographical variations in heterogeneous populations.

Limitations

One of the limitations of the present study is a small sample size. In addition, the retrospective nature of the study might confine the risk assessment for different types of gingival lesions due to the lack of data from the patients' records. Further multicenter, prospective studies with larger sample sizes are needed to better understand the characteristics, frequency distribution and risk indicators of gingival lesions.

Conclusions

Inadequate or unclear classification criteria complicate the clinician diagnosis, treatment and referral. Hence, comprehensive diagnostic codes are important tools for the clinician to be specific in categorizing the disease. Within the limitations of the present study, reactive processes were the most frequent, while genetic/developmental disorders

were the least prevalent. The implementation of the current classification in diagnostic settings provides a more holistic approach by categorizing gingival lesions into 6 different types and further subtypes in comparison with the older classification system, which included the majority of lesions under the "not otherwise specified" category.

Ethics approval and consent to participate

Ethical clearance was obtained from the institutional ethics committee at All India Institute of Medical Sciences, New Delhi, India (IEC-720/04.10.2019, RP-31/2019).

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Vandana Gupta https://orcid.org/0000-0002-9299-801X
Harpreet Kaur https://orcid.org/0000-0002-1646-1253
Deepika Mishra https://orcid.org/0000-0002-7624-1156
Vikender Singh Yadav https://orcid.org/0000-0001-9359-153X
Sunny Kala https://orcid.org/0000-0001-6170-3736

References

1. Holmstrup P, Plemons J, Meyle J. Non-plaque-induced gingival diseases. *J Periodontol*. 2018;89(Suppl 1):S28–S45. doi:10.1002/JPER.17-0163
2. Holmstrup P, Schiøtz AW, Westergaard J. Effect of dental plaque control on gingival lichen planus. *Oral Surg Oral Med Oral Pathol*. 1990;69(5):585–590. doi:10.1016/0030-4220(90)90241-j
3. Holmstrup P. Non-plaque-induced gingival lesions. *Ann Periodontol*. 1999;4(1):20–31. doi:10.1902/annals.1999.4.1.20
4. Carbone M, Broccoletti R, Gambino A, et al. Clinical and histological features of gingival lesions: A 17-year retrospective analysis in a northern Italian population. *Med Oral Patol Oral Cir Bucal*. 2012;17(4):e555–e561. doi:10.4317/medoral.17809
5. Oliver RJ, Sloan P, Pemberton MN. Oral biopsies: Methods and applications. *Br Dent J*. 2004;196(6):329–333, quiz 362. doi:10.1038/sj.bdj.4811075
6. Vidyanath S, Shameena PM, Johns DA, Shivashankar VY, Sudha S, Varma S. Reactive hyperplasic lesions of the oral cavity: A survey of 295 cases at a Tertiary Health Institution in Kerala. *J Oral Maxillofac Pathol*. 2015;19(3):330–334. doi:10.4103/0973-029X.174614
7. Rich AM, Seo B, Parachuru V, Hussaini HM. The nexus between periodontics and oral pathology. *Periodontol 2000*. 2017;74(1):176–181. doi:10.1111/prd.12197
8. Buchner A, Shnaiderman-Shapiro A, Vered M. Relative frequency of localized reactive hyperplastic lesions of the gingiva: A retrospective study of 1675 cases from Israel. *J Oral Pathol Med*. 2010;39(8):631–638. doi:10.1111/j.1600-0714.2010.00895.x
9. Al-Mobeeriek A, AlDosari AM. Prevalence of oral lesions among Saudi dental patients. *Ann Saudi Med*. 2009;29(5):365–368. doi:10.4103/0256-4947.55166
10. Kamath KP, Vidya M, Anand PS. Biopsied lesions of the gingiva in a southern Indian population – a retrospective study. *Oral Health Prev Dent*. 2013;11(1):71–79. doi:10.3290/j.ohpd.a29378
11. Koo MG, Lee SH, Han SE. Pyogenic granuloma: A retrospective analysis of cases treated over a 10-year. *Arch Craniofac Surg*. 2017;18(1):16–20. doi:10.7181/acfs.2017.18.1.16
12. Zarei MR, Chamani G, Amanpoor S. Reactive hyperplasia of the oral cavity in Kerman province, Iran: A review of 172 cases. *Br J Oral Maxillofac Surg*. 2007;45(4):288–292. doi:10.1016/j.bjoms.2006.10.001
13. Buchner A, Hansen LS. The histomorphologic spectrum of peripheral ossifying fibroma. *Oral Surg Oral Med Oral Pathol*. 1987;63(4):452–461. doi:10.1016/0030-4220(87)90258-1
14. Manjunatha BS, Sutariya R, Nagamahita V, Dholia B, Shah V. Analysis of gingival biopsies in the Gujarati population: A retrospective study. *J Cancer Res Ther*. 2014;10(4):1088–1092. doi:10.4103/0973-1482.137929
15. daSilva FC, Piazzetta CM, Torres-Pereira CC, Schussel JL, Amenábar JM. Gingival proliferative lesions in children and adolescents in Brazil: A 15-year-period cross-sectional study. *J Indian Soc Periodontol*. 2016;20(1):63–66. doi:10.4103/0972-124X.168493
16. Alblowi JA, Binmadi NO. Histopathologic analysis of gingival lesions: A 20-year retrospective study at one academic dental center. *J Clin Exp Dent*. 2018;10(6):e561–e566. doi:10.4317/jced.54766
17. Ariyoshi Y, Shimahara M, Omura K, et al. Epidemiological study of malignant tumors in the oral and maxillofacial region: Survey of member institutions of the Japanese Society of Oral and Maxillofacial Surgeons, 2002. *Int J Clin Oncol*. 2008;13(3):220–228. doi:10.1007/s10147-007-0756-9
18. Hernández-Ríos P, Espinoza I, Salinas M, Rodríguez-Castro F, Baeza M, Hernández M. Distribution of biopsied non plaque-induced gingival lesions in a Chilean population according to the classification of periodontal diseases. *BMC Oral Health*. 2018;18(1):112. doi:10.1186/s12903-018-0567-6
19. Barasch A, Gofa A, Krutchkoff DJ, Eisenberg E. Squamous cell carcinoma of the gingiva. A case series analysis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1995;80(2):183–187. doi:10.1016/s1079-2104(05)80200-8
20. Krutchkoff DJ, Chen JK, Eisenberg E, Katz RV. Oral cancer: A survey of 566 cases from the University of Connecticut Oral Pathology Biopsy Service, 1975–1986. *Oral Surg Oral Med Oral Pathol*. 1990;70(2):192–198. doi:10.1016/0030-4220(90)90118-c

Clinical and antioxidant efficacy of 4% mangosteen gel as a local drug delivery in the treatment of chronic periodontitis: A placebo-controlled, split-mouth trial

Vinayaka Ambujakshi Manjunatha[1,A–F], Gayathri Gunjiganur Vemanaradhya[2,A,E,F], Triveni Mavinakote Gowda[2,E]

[1] Department of Periodontology, PMNM Dental College and Hospital, Bagalkot, India
[2] Department of Periodontology, Bapuji Dental College and Hospital, Davangere, India

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):111–119

Address for correspondence
Vinayaka Ambujakshi Manjunatha
E-mail: dravidavinu@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
The authors would like to acknowledge Professor Thimmasetty Jutur, the Head of the Department of Pharmaceutics at Bapuji Pharmacy College, Davangere, India, for his assistance in the preparation of the mangosteen gel, Mr. Varun Eranna from the Research Faculty of the University of Trans-Disciplinary Health Sciences and Technology, Bangalore, India, as well as the participants of the clinical trial.

Received on April 20, 2021
Reviewed on June 17, 2021
Accepted on June 21, 2021

Published online on March 31, 2022

Cite as
Manjunatha VA, Vemanaradhya GG, Gowda TM. Clinical and antioxidant efficacy of 4% mangosteen gel as a local drug delivery in the treatment of chronic periodontitis: A placebo-controlled, split-mouth trial. *Dent Med Probl.* 2022;59(1):111–119. doi:10.17219/dmp/139198

DOI
10.17219/dmp/139198

Abstract

Background. Currently, the knowledge on the clinical effect of 4% mangosteen gel as a local drug delivery, adjunctive to non-surgical periodontal therapy, on the gingival crevicular fluid (GCF) total antioxidant capacity (TAOC) levels in chronic periodontitis patients is limited.

Objectives. The aim of the study was to evaluate and compare the efficacy of 4% mangosteen gel and a placebo gel as an adjunct to scaling and root planing (SRP) on clinical and biochemical parameters in chronic periodontitis patients.

Material and methods. A total of 50 test sites from 25 patients with Stage II Grade B periodontitis were randomly divided into 2 groups. The experimental group was treated with SRP followed by a single subgingival application of 4% mangosteen gel, while the control group was treated with SRP followed by a single subgingival application of a placebo gel. Clinical parameters, including the plaque index (PI), the gingival bleeding index (GBI), the probing depth (PD), the relative attachment level (RAL), as well as biochemical parameters, i.e., the GCF TAOC levels, were evaluated using an ABTS (2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) antioxidant assay kit at baseline (D0) and at 3 months (D3).

Results. The full-mouth PI and GBI values were significantly lower at D3 in comparison with D3. The experimental sites showed a significantly greater reduction in the PD and RAL scores as compared to control, and the GCF TAOC levels revealed a substantial rise throughout the study period, reversing from negative values at D0 to positive values at D3 in the experimental group.

Conclusions. Traditional SRP with the adjunctive local delivery of 4% mangosteen gel demonstrated an added benefit in improving clinical and biochemical parameters, and thereby encouraging the use of the mangosteen gel in clinical practice for the management of moderate chronic periodontitis.

Keywords: periodontitis, mangosteen, root planing, scaling

Introduction

One of the primary etiological factors for periodontitis is a microbial shift, which is always associated with a reduction in the number of beneficial symbionts and/or a rise in the number of pathogenic microorganisms in the ecosystem of the subgingival biofilm.[1] These pathogens disrupt the interaction between the host and the oral microbiome, eventually favoring the onset and progression of periodontal disease. Various cytokines, such as interleukin 8 (IL-8) and tumor necrosis factor alpha (TNF-α), are produced by pathogenic Gram-negative, anaerobic or facultative bacteria within the subgingival biofilm, further causing a surge in polymorphonucleocyte (PMN) activity as part of the primary immune response.[1,2]

Polymorphonucleocytes fight against microbes through the release of enzymes that are involved in the breakdown of proteins into smaller polypeptides or amino acids (proteolysis), as well as free radicals or reactive oxygen species (ROS) by an aerophilous surge. In order to maintain a homeostatic environment or to suppress the pathogenicity of microorganisms, ROS are detoxified and modified by the antioxidant system to form less reactive species.[1–3]

The traditional non-surgical periodontal therapy includes mechanical debridement by scaling and root planing (SRP). However, the efficacy of this treatment is limited due to inaccessibility in areas such as furcations, grooves, concavities, and deep periodontal pockets.[3–5]

To overcome this limitation, the local drug delivery (LDD) has been introduced. It has gained a lot of popularity due to certain advantages, such as a high and sustained local concentration of the drug without applying large doses, thus minimizing toxicity in comparison with systemic therapy.[6] Although various agents are being utilized as LDD, the need for more biocompatible and economical agents has consistently inspired clinicians to move toward herbal substances.

Pharmacologically active phytochemicals obtained from plants have been widely identified as useful aids for the prevention, treatment and maintenance therapy of periodontal disease.[7] *Garcinia mangostana*, commonly referred to as mangosteen, also known as "the queen of fruits", belongs to the *Guttiferae* family. It contains diverse bioactive compounds, such as camphene, garcinones A, B and C, sesquiterpenoids, gartanin, and tannins. The significant characteristics of mangosteen include antioxidant, antimicrobial and anti-inflammatory properties.[8–12]

The LLD of the mangosteen gel following SRP has been shown to result in large decreases in the probing depth (PD), gingival index (GI) and gingival bleeding index (GBI) values, as well as improvement in clinical epithelial attachment in chronic periodontitis patients.[9–12]

Total antioxidant capacity (TAOC) is the degree of the magnitude of free radicals rummaged by a test solution, expressed as percentage and used to gauge the antioxidant capacity of a biological medium, like saliva, serum and gingival crevicular fluid (GCF).[1,2]

Gingival crevicular fluid is considered the most appropriate medium for investigating inflammatory biomarkers to evaluate oxidative stress in the supporting tissues of the periodontium.[1,2,13] Previous studies have shown improvement in the periodontal status and the GCF TAOC levels in adult periodontitis patients, following non-surgical periodontal therapy alone.[13] So far, only the antimicrobial potential of the mangosteen pericarp gel has been explicitly assessed, but its antioxidant impact in the treatment of chronic periodontitis has not been estimated. Hence, the aim of this study was to evaluate and compare the efficacy of the LDD of 4% mangosteen gel as an adjunct to non-surgical periodontal therapy on the GCF TAOC levels and clinical parameters in patients with chronic periodontitis.

Material and methods

Preliminary plan and moral statement

This clinico-biochemical study was carried out as a prospective, double-blinded, randomized, controlled, split-mouth trial. It was approved by the institutional review board (IRB) at Bapuji Dental College and Hospital, Davangere, India (No. BDCH/Exam 467/2018–2019). The study was performed in compliance with the ethical standards established by the World Medical Association (WMA) in the Declaration of Helsinki. Each patient was given a detailed verbal and written description of the study, and a signed consent form was obtained. The flow chart of the study design is presented in Fig. 1.

The split-mouth design and randomization were used in the present study to avoid inter-subject variability and bias. The selection of the test sites for both groups was also standardized at baseline to ensure reliable results.

Inclusion and exclusion criteria[13]

The patients were designated from the ambulatory care unit, Department of Periodontology, Bapuji Dental College and Hospital, according to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Patients within the age range of 35–60 years for both genders, with Stage II Grade B periodontitis (moderate periodontitis) were included. Patients having a minimum of 2 sites with PD ≤ 5 mm and clinical attachment loss (CAL) ≤4 mm with horizontal bone loss at 2 different quadrants, and requiring non-surgical treatment were considered as eligible for the study.

Patients with habits, like smoking, tobacco chewing or alcohol consumption, having uncontrolled diabetes, hypertension or immunocompromised conditions, pregnant women or lactating mothers, non-compliant or physically challenged people, and those who were not able to maintain oral hygiene or be followed up at a recall visit were excluded from the study.

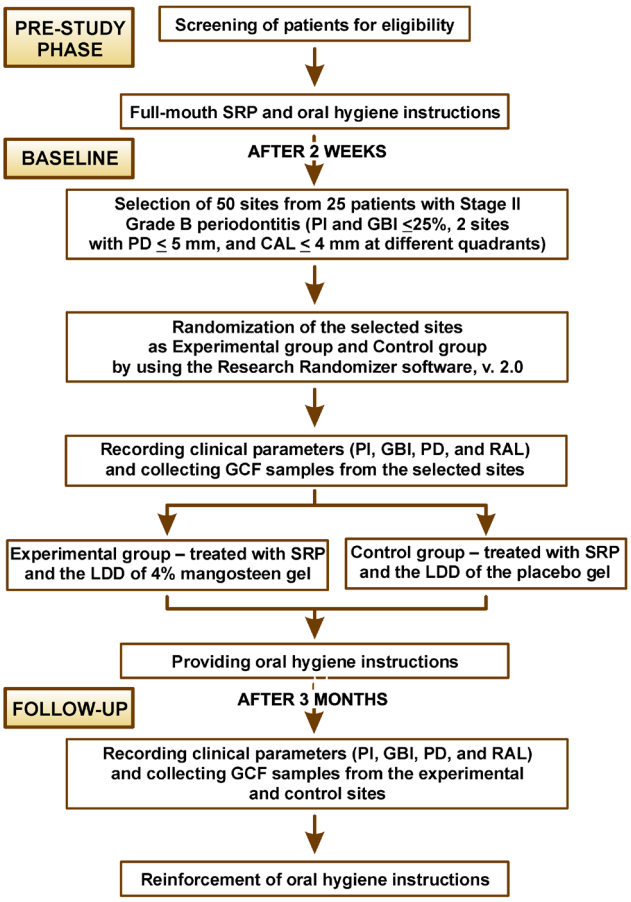

Fig. 1. Study design

SRP – scaling and root planing; PI – plaque index; GBI – gingival bleeding index; PD – probing depth; CAL – clinical attachment loss; RAL – relative attachment level; GCF – gingival crevicular fluid; LDD – local drug delivery.

Sample size calculation[14]

The sample size was calculated using the following equation (Equation 1):

$$N = \left[\frac{Z_1 - \alpha/2 + Z_1 - \beta}{\mu_A - \mu_B} \right]^2 \quad (1)$$

where:
N – sample size;
Z_1 – Z-value;
α – level of significance;
β – level of power;
$\mu_A - \mu_B$ – mean difference between the samples.

As per the calculation, at least 40 sites were needed to provide a power of 80% and to detect differences in the mean PD values between 2 study groups by the end of a 3-month period. A total of 50 sites were recruited to compensate for a 20% dropout rate.

Estimation of clinical parameters

Following the initial screening, supragingival full-mouth SRP was performed in all patients. After 2 weeks, only 25 patients who fulfilled the inclusion and exclusion criteria, with the full-mouth PI and GBI values ≤25% were selected. Both at baseline (D0) and at 3 months after treatment (D3), the PI,[15] GBI,[16] PD, and RAL values were recorded, and GCF samples were collected for the evaluation of the TAOC levels by a blinded examiner.

Randomization

The selected 50 sites from 25 patients were randomly assigned by means of the Research Randomizer software, v. 2.0 (https://www.randomizer.org/).

Groups

The selected sites for each group were managed with the allocated treatment plan by a qualified clinician. The control group sites were treated with SRP followed by a single application of the placebo gel. The experimental group sites were treated with SRP followed by a single application of 4% mangosteen gel.

Preparation of 4% *Garcinia mangostana* gel and the placebo gel

Pure mangosteen powder was obtained from Tamil Nadu Agricultural University, Coimbatore, India. The 4% mangosteen gel was made in the proportions presented by Rassameemasmaung et al.[11]

The concentration of the gel was adjusted according to the data obtained from a study by Mahendra et al.[9] A homogenizer was used for homogenizing all ingredients to produce the gel (Fig. 2). The placebo gel was prepared in the same manner, excluding the active ingredient (the mangosteen powder) and maintaining the same physical properties, such as color and taste (Fig. 3). Both 4% mangosteen gel and the placebo gel were preserved at 4°C during the course of the study.[9]

Collection of GCF samples[13]

After isolating the designated teeth, a standardized measure of 5 µL GCF from each test site was collected by placing calibrated, black color-coded 1–5-microliter volumetric microcapillary pipettes (Sigma-Aldrich, Burlington, USA) with the use of the extracrevicular (unstimulated) method. The collected GCF samples were moved to sterile Eppendorf tubes with 50 µL of alkaline phosphate buffered saline (PBS) and tagged according to the tooth number assigned to the chosen test group. Then, they were sealed firmly and immediately transported to the research laboratory, where they were stored at –80°C till the time of assay.

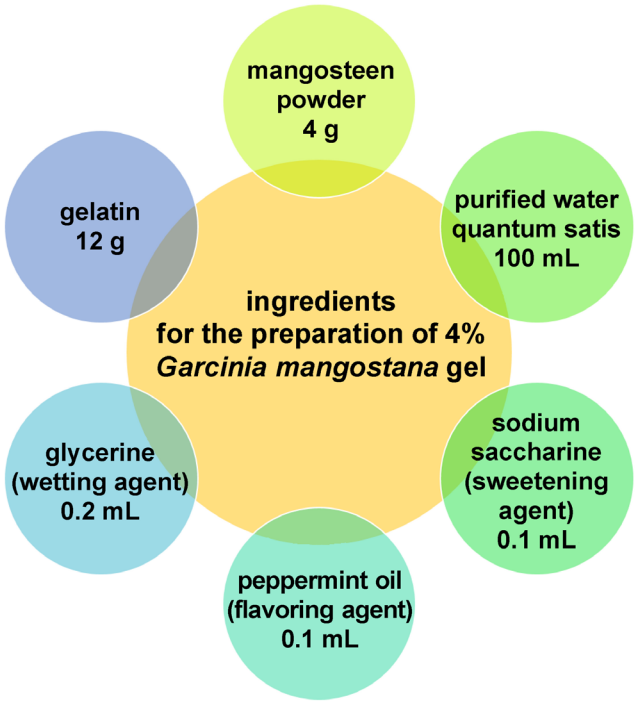

Fig. 2. Ingredients for the preparation of 4% mangosteen gel

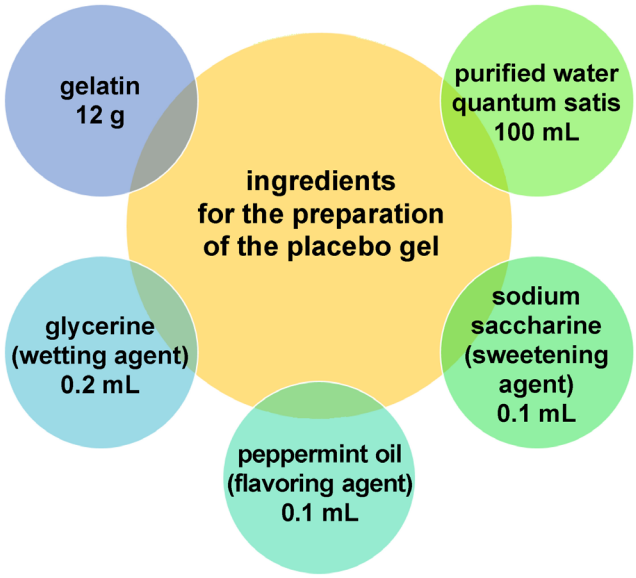

Fig. 3. Ingredients for the preparation of the placebo gel

Scaling and root planing protocol[13]

All the selected patients underwent thorough subgingival SRP with the use of ultrasonic scalers (Woodpecker India, New Delhi, India). Root planing was conducted using either 4R/4L or 2R/2L Columbia universal curettes (Hu-Friedy, Chicago, USA).

Intracrevicular administration of 4% mangosteen gel and the placebo gel

For standardization, both 4% mangosteen gel (4 g/100 mL) and the placebo gel were loaded into a hypodermic syringe with a 24-gauge angulated blunt needle and delivered to the experimental and control sites, respectively.

The tip of the needle was slowly slid over the tooth surface down to the base of the pocket, and the allocated gel was injected into the deepest part of the pocket at both experimental and control sites. While extruding the material, the needle was gradually withdrawn till it reached the upper portion of the pocket and the excess gel was removed. The selected sites were sealed with a periodontal dressing to prevent the ingress of oral fluids, which was then removed after 1 week.[13,17]

Biochemical evaluation

An ABTS (2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) antioxidant assay kit (Zen-Bio Inc., Durham, USA) was used to determine the GCF TAOC levels at the selected sites. The collected GCF samples were added to the wells of microtiter-coated plates with a pipette. The plates were sealed and incubated for 2 h at room temperature (18–25°C). The reagents were added as per the manufacturer's instructions. Color development was monitored every 5 min. Positive wells turned blue in color. The determination of optical density in each well was performed using a microplate reader (Molecular Devices, San Jose, USA) at an absorbance of 405 nm.

Statistical analysis

The data obtained from the clinical and biochemical evaluation is presented as mean and standard deviation ($M \pm SD$). The PI, GBI, PD, and RAL parameters were analyzed using Student's t test, one-way analysis of variance (ANOVA) and Šidák's post hoc test for pairwise comparisons. The GCF TAOC levels were analyzed using the Mann–Whitney U test for pairwise comparisons, Student's t test and the Kruskal–Wallis test. For all tests, $p \leq 0.001$ was considered statistically significant.

Results

Clinical parameters

A significant reduction ($p \leq 0.001$) was observed in the PI, GBI (Fig. 4 and Fig. 5, respectively), PD, and RAL (Tables 1,2) scores in both groups at D3 as compared to D0.

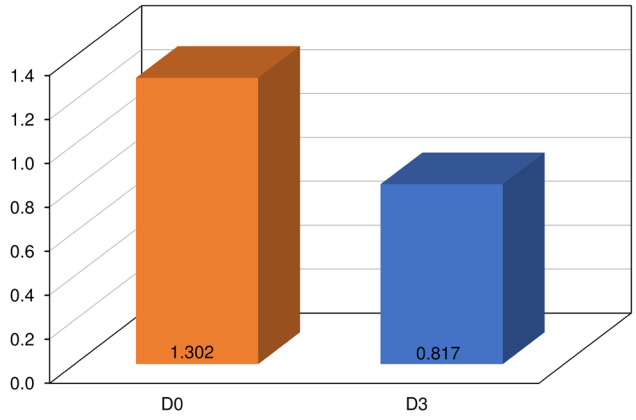

Fig. 4. Comparison of the mean plaque index (PI) scores at baseline (D0) and at 3 months post-treatment (D3)

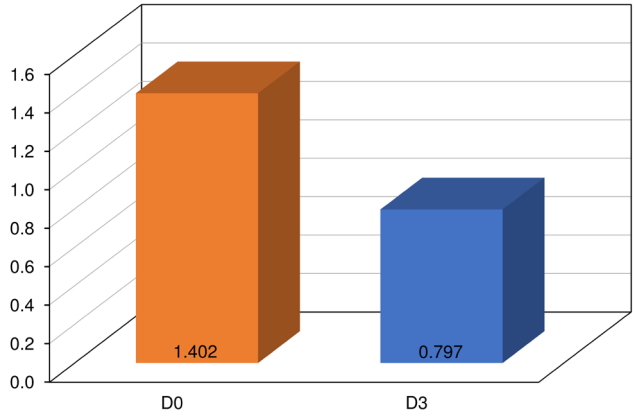

Fig. 5. Comparison of the mean gingival bleeding index (GBI) scores at baseline (D0) and at 3 months post-treatment (D3)

Probing depth (PD)

Intragroup comparison of the PD scores at D0 and D3 in the control and experimental groups

In the control group, the mean PD score at D0 was 5.630 ±0.928 mm, and then it decreased to 4.200 ±0.887 mm at D3. The difference between the pre- and post-treatment mean PD scores was 1.430 ±0.041 mm ($p < 0.001$; $f = 0.790$).

In the experimental group, the mean PD score at D0 was 5.730 ±0.828 mm, which was reduced to 2.870 ±0.900 mm after treatment at D3. The difference between the pre- and post-treatment mean PD scores was 2.860 ±0.072 mm ($p < 0.001$; $f = 1.350$) (Table 1).

Intergroup comparison of the PD scores at D0 and D3 in the control and experimental groups

At D0, the mean PD score was lower in the control group (5.630 ±0.928 mm) than in the experimental group (5.730 ±0.828 mm). The difference in the mean PD scores between the control and experimental sites was –0.100 ±0.100 mm, which was not statistically significant. At D3, the mean PD score was higher in the control group (4.200 ±0.887 mm) as compared to the experimental group (2.870 ±0.900 mm). The difference in the mean PD scores between the control and experimental sites was 1.330 ±0.013 mm ($p < 0.001$) (Table 2).

Table 1. Comparison of the probing depth (PD), the relative attachment level (RAL) and the gingival crevicular fluid (GCF) total antioxidant capacity (TAOC) levels between baseline (D0) and 3 months (D3) for both the control and experimental sites

Parameter	Control sites					Experimental sites				
	D0	D3	D3 vs. D0‡	*p*-value#	*f*-value†	D0	D3	D3 vs. D0‡‡	*p*-value#	*f*-value††
PD [mm]	5.630 ±0.928	4.200 ±0.887	1.430 ±0.041	<0.001*	0.790	5.730 ±0.828	2.870 ±0.900	2.860 ±0.072	<0.001*	1.350
RAL [mm]	7.630 ±1.351	6.730 ±1.112	0.900 ±0.239	<0.001*	1.000	8.030 ±1.098	5.930 ±1.230	2.100 ±0.132	<0.001*	2.340
GCF TAOC level [μM]	–0.294 ±0.901	–0.178 ±0.145	–0.116 ±0.756	>0.001	41.261	–0.374 ±0.215	0.041 ±0.200	–0.415 ±0.015	<0.001*	41.641

Data presented as mean ± standard deviation (*M* ±*SD*). ‡ Šidák's post hoc test; ‡‡ Mann–Whitney *U* test; # Student's *t* test; † ANOVA; †† Kruskal–Wallis test; * statistically significant.

Table 2. Comparison of the probing depth (PD), the relative attachment level (RAL) and the gingival crevicular fluid (GCF) total antioxidant capacity (TAOC) levels between the control and experimental sites at both baseline (D0) and 3 months (D3)

Time of assessment	PD [mm]				RAL [mm]				GCF TAOC level [μM]			
	control sites	experimental sites	D3 vs. D0‡	*p*-value#	control sites	experimental sites	D3 vs. D0‡	*p*-value#	control sites	experimental sites	D3 vs. D0‡‡	*p*-value#
D0	5.630 ±0.928	5.730 ±0.828	–0.100 ±0.100	>0.001	7.630 ±1.351	8.030 ±1.098	–0.400 ±0.253	>0.001	–0.294 ±0.901	–0.374 ±0.215	0.080 ±0.686	<0.001*
D3	4.200 ±0.887	2.870 ±0.900	1.330 ±0.013	<0.001*	6.730 ±1.112	5.930 ±1.230	0.800 ±0.118	<0.001*	–0.178 ±0.145	0.041 ±0.200	–0.219 ±0.055	<0.001*

Data presented as *M* ±*SD*. ‡ Šidák's post hoc test; ‡‡ Mann–Whitney U test; # Student's t test; * statistically significant.

Relative attachment level (RAL)

Intragroup comparison of the RAL scores at D0 and D3 in the control and experimental groups

In the control group, the mean RAL score at D0 was 7.630 ±1.351 mm, and then it decreased to 6.730 ±1.112 mm at D3. The difference between the pre- and post-treatment mean RAL scores was 0.900 ±0.239 mm ($p < 0.001$; $f = 1.000$).

In the experimental group, the mean RAL score at D0 was 8.030 ±1.098 mm, which was reduced to 5.930 ±1.230 mm after treatment at D3. The difference between the pre- and post-treatment mean RAL scores was 2.100 ±0.132 mm ($p < 0.001$; $f = 2.340$) (Table 1).

Intergroup comparison of the RAL scores at D0 and D3 in the control and experimental groups

At D0, the mean RAL score was lower in the control group (7.630 ±1.351 mm) than in the experimental group (8.030 ±1.098 mm). The difference in the mean RAL scores between the control and experimental sites was −0.400 ±0.253 mm, which was not statistically significant. At D3, the mean RAL score was higher in the control group (6.730 ±1.112 mm) as compared to the experimental group (5.930 ±1.230 mm). The difference in the mean RAL scores between the control and experimental sites was 0.800 ±0.118 ($p < 0.001$) (Table 2).

The intra-examiner reliability was checked using Cohen's kappa coefficient, which amounted to 0.82, indicating good precision and agreement.

Biochemical parameters

Gingival crevicular fluid (GCF) total antioxidant capacity (TAOC) level

Intragroup comparison of the GCF TAOC levels at D0 and D3 in the control and experimental groups

In the control group, the mean GCF TAOC level at D0 was −0.294 ±0.901 μM, and then it increased to −0.178 ±0.145 μM at D3. The difference between the pre- and post-treatment mean GCF TAOC levels was −0.116 ±0.756 μM, which was not statistically significant.

In the experimental group, the mean GCF TAOC level at D0 was −0.374 ±0.215 μM, which rose to 0.041 ±0.200 μM after treatment at D3. The difference between the pre- and post-treatment mean GCF TAOC levels was −0.415 ±0.015 μM ($p < 0.001$; $f = 41.641$) (Table 1).

Intergroup comparison of the GCF TAOC levels at D0 and D3 in the control and experimental groups

At D0, the mean GCF TAOC level was found to be higher in the control group (−0.294 ±0.901 μM) than in the experimental group (−0.374 ±0.215 μM). The difference in the mean GCF TAOC levels between the control and experimental sites was 0.080 ±0.686 μM ($p < 0.001$). At D3, the mean GCF TAOC level was lower in the control group (−0.178 ±0.145 μM) as compared to the experimental group (0.041 ±0.200 μM). The difference in the mean GCF TAOC levels between the control and experimental sites was −0.219 ±0.055 μM ($p < 0.001$) (Table 2).

Correlation between clinical parameters and GCF TAOC levels

At 3-month follow-up, a significant positive correlation was observed between the GCF TAOC levels and one of the clinical parameters (RAL) in the experimental group (the 4% mangosteen gel group) when compared to the control group (the placebo gel group) (Fig. 6).

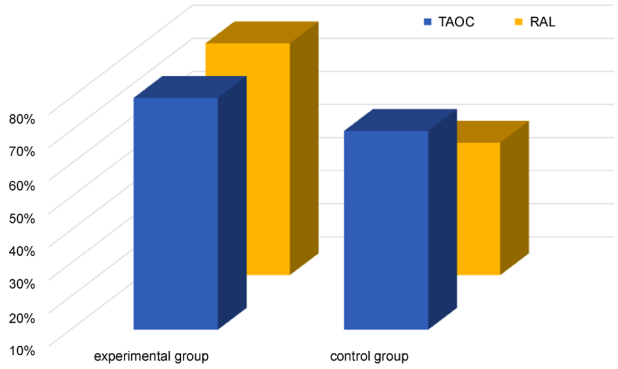

Fig. 6. Positive correlation between relative attachment level (RAL) and the gingival crevicular fluid (GCF) total antioxidant capacity (TAOC) level

Discussion

The authors of the present study hypothesized that the LDD of 4% mangosteen gel as an adjunct to SRP would show superior outcomes as compared to SRP with the placebo gel in terms of clinical efficacy and antioxidant potential, which is essential in the management of periodontitis patients. Differences in the study outcomes between the experimental and control groups were found to be statistically significant, with the 4% mangosteen gel group exhibiting better performance. Thus, the formulated hypothesis was confirmed. The adjunctive use of a subgingival LDD with gel formulations for the management of periodontitis have been highly popularized due to their less invasiveness and their ability to access deep-seated sites, such as furcations and invaginations.[13,18]

Recently, different vegetative antimicrobials have acquired extraordinary significance in the arena of preventive periodontics, as they have no aftereffects.[9–11] Thus, they are being extensively used as an unconventional

alternative to systemic antimicrobials, which have disadvantages, like hypersensitivity reactions, gastrointestinal intolerance and the development of bacterial resistance.[19,20] These downsides can be evaded with the local application of antimicrobial medications in the subgingival environment, achieving an adequate concentration of the drug for a longer duration of time.[21] The adjunctive use of an antimicrobial LDD has been observed to produce a superior effect, with a decrease in PD and gingival inflammation.[18]

Mangosteen is a marvelous fruit; its pericarp is commonly utilized in the Middle East for the therapy of diarrhea, and skin and chronic wound infections.[7] Since ancient times, this natural product has been a fundamental element in Chinese medicine and Ayurveda.[22] It appears to have antifungal, anticytotoxic, antiviral, antibacterial, antihistamine, antioxidant, and anti-inflammatory properties.[8–10] The leaves and bark of the mangosteen tree are utilized to produce chewing sticks as an astringent in oral care in some African nations.[23] The mangosteen pericarp extract has exhibited antibacterial properties against numerous pathogenic microbes.[24–27] Previous studies have indicated that the mangosteen pericarp extract is effective against *Streptococcus mutans* at a minimum inhibitory concentration (MIC) – the lowest concentration of a chemical substance (usually a drug) that prevents bacteria from growing significantly – of 0.625 μg/mL.[11]

Its active components are xanthone derivatives, such as α-, β- and γ-mangostin, gartinin, and 1- and 3-isomangostin. Among these, α-mangostin has the strongest antibacterial effect.[28] Studies have also demonstrated that the mangosteen extract has low toxicity when applied topically.[29,30] Alpha-mangostin, when administered orally to rats at a high dose (1.5 g/kg body weight) to test its hepatotoxicity, demonstrated a lowered serum glutamate pyruvate transferase activity for a period of 12 h, indicative of its safety with a topical application.[31]

The ethanolic extract (80%) from the pericarp of mangosteen inhibited the growth of *Porphyromonas gingivalis (P. gingivalis)*, the main periodontopathic bacteria, at a MIC of 3.91 mg/mL[8,9] and showed positive results in the reduction of clinical inflammation in chronic periodontitis patients with a dosage of 4% mangosteen gel.[10] Hence, the preparation of 4% mangosteen gel preparation was considered in the present study.

Total antioxidant capacity is an important biochemical marker in determining the cumulative action of antioxidants present in body fluids.[1,2,18] The extracrevicular (unstimulated) method with the aid of microcapillary pipettes was used for GCF sampling in an attempt to minimize trauma to the gingival tissues,[13,32] preventing bleeding and the contamination of the GCF sample.

At the end of the 3-month study, both groups showed a statistically significant reduction in PI, GBI, PD, and RAL. This may be attributed to the thorough removal of local factors during the pre-study phase, and the selection of patients with PI and GBI <25%, along with the reinforcement of oral hygiene instructions at baseline.

The full-mouth PI scores reflect the oral hygiene status of individual patients. The significant reduction in the PI scores from D0 to D3 in the present study was similar to that observed by Mahendra et al.[9]

Bleeding associated with inflammation may be due to structural alterations in the vessel walls and environs, which results in increased capillary fragility and permeability, predisposing to bleeding upon probing.[16] In our study, significant improvement in the GBI scores occurred at the end of the 3-month period. This improvement confirms the better oral hygiene status of the patients, prolonging the effect of the therapy, which is attributed to the Hawthorne effect.[8–10] The obtained results are in accordance with those of Rassameemasmaung et al., who showed a similar reduction in the GBI scores upon the application of 4% mangosteen gel.[11]

Similar to other studies,[8–10] the experimental sites (the 4% mangosteen gel group) showed a greater reduction in the PD and RAL scores as compared to the control sites (the placebo gel group). The obtained results may be due to the resolution of gingival inflammation, causing the shrinkage and reattachment of the connective tissue fibers, along with the downregulation of inflammatory mediators, given the short duration of this study.[13]

Periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans* or *P. gingivalis*, implicate oxidative stress, thus launching the production of free radicals and ROS in higher concentrations, which results in the destruction of the periodontal tissues, either by degrading the ground substance or by releasing collagenases and various inflammatory mediators.[2,4,5]

It is noteworthy that although both the study groups showed statistically significant increases in the GCF TAOC levels, a slightly greater increase in the mean GCF TAOC level was observed in the experimental group (the 4% mangosteen gel group) in comparison with the control group after 3 months. This may be due to the antioxidant effect of mangosteen, along with the scavenging of free radicals by the enzymatic mechanism of superoxide dismutase.[33]

At baseline, both groups demonstrated a statistically significant association between the GCF TAOC levels and changes in clinical parameters. This substantiates the assumption that cases of periodontal disease are associated with low GCF TAOC levels due to an increase in the level of ROS.[12]

A statistically significant positive correlation between RAL and the GCF TAOC levels was also observed in the experimental group (the 4% mangosteen gel group) at a 3-month follow-up. This is attributed to the direct effect of mangosteen on the upregulation of the GCF TAOC levels, which brought improvement in clinical parameters, hence proving its antioxidant potential.[12]

This is the first clinical trial to compare the efficacy of both 4% mangosteen gel and a placebo gel as adjuncts to SRP by evaluating changes in clinical parameters and the GCF TAOC levels upon their subgingival LDD. However, the present study recruited a small number of patients who were followed up for only 3 months. Hence, further randomized, controlled clinical trials need to be considered with larger sample sizes and longer follow-up periods, employing different modes of LDD and greater concentrations of mangosteen, with various antioxidant biomarkers to obtain stronger outcomes.

Conclusions

This clinic-biochemical trial demonstrated that the local delivery of 4% mangosteen gel into the periodontal pockets of chronic periodontitis patients significantly reduced the mean PI, GBI and PD scores, and gain in the RAL scores. It also demonstrated a significant increase in the GCF TAOC levels at the end of the 3-month period, proving the antioxidant efficacy of 4% mangosteen gel. This can provide a new direction in the field of periodontal therapy. However, long-term, multicenter, randomized, controlled clinical trials are required in order to further understand the clinical and microbiological profile of patients with chronic periodontitis after the application of mangosteen gel as LDD, and also to compare this treatment protocol with other established and clinically proven standard drugs.

Ethics approval and consent to participate

The study was approved by the institutional review board (IRB) at Bapuji Dental College and Hospital, Davangere, India (No. BDCH/Exam 467/2018–2019). It was performed in compliance with the ethical standards established by the World Medical Association (WMA) in the Declaration of Helsinki. All participants provided written informed consent prior to the investigations.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Vinayaka Ambujakshi Manjunatha https://orcid.org/0000-0002-1255-7944
Gayathri Gunjiganur Vemanaradhya https://orcid.org/0000-0002-7791-4113
Triveni Mavinakote Gowda https://orcid.org/0000-0003-3207-0137

References

1. Sharma A, Sharma S. Reactive oxygen species and antioxidants in periodontics: A review. *Int J Dent Clin*. 2011;3(2):44–47.
2. Dahiya P, Kamal R, Gupta R, Bhardwaj R, Chaudhary K, Kaur S. Reactive oxygen species in periodontitis. *J Indian Soc Periodontol*. 2013;17(4):411–416. doi:10.4103/0972-124X.118306
3. Mombelli A. Microbial colonization of the periodontal pocket and its significance for periodontal therapy. *Periodontol 2000*. 2018;76(1):85–96. doi:10.1111/prd.12147
4. Brittan JL, Sprague SV, Macdonald EL, Love RM, Jenkinson HF, West NX. In vivo model for microbial invasion of tooth root dentinal tubules. *J Appl Oral Sci*. 2016;24(2):126–135. doi:10.1590/1678-775720150448
5. Colombo AV, Silva CM, Haffajee A, Vieira Colombo AP. Identification of oral bacteria associated with crevicular epithelial cells from chronic periodontitis lesions. *J Med Microbiol*. 2006;55(Pt 5):609–615. doi:10.1099/jmm.0.46417-0
6. Nair SC, Anoop KR. Intraperiodontal pocket: An ideal route for local antimicrobial drug delivery. *J Adv Pharm Technol Res*. 2012;3(1):9–15. doi:10.4103/2231-4040.93558
7. Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. *Curr Med Chem*. 2004;11(11):1451–1460. doi:10.2174/0929867043365107
8. Hendiani I, Hadidjah D, Susanto A, Setia Pribadi IM. Inhibitory and bactericidal power of mangosteen rind extract towards *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* (laboratory test). *Padjadjaran J Dent*. 2016;28(2):75–80. doi:10.24198/pjd.vol28no2.13605
9. Mahendra J, Mahendra L, Svedha P, Cherukuri S, Romanos GE. Clinical and microbiological efficacy of 4% *Garcinia mangostana* L. pericarp gel as local drug delivery in the treatment of chronic periodontitis: A randomized, controlled clinical trial. *J Investig Clin Dent*. 2017;8(4). doi:10.1111/jicd.12262
10. Hendiani I, Hadidjah D, Susanto A, Setia Pribadi IM. The effectiveness of mangosteen rind extract as additional therapy on chronic periodontitis (clinical trials). *Padjadjaran J Dent*. 2017;29(1):64–70. doi:10.24198/pjd.vol29no1.12986
11. Rassameemasmaung S, Sirikulsathean A, Amornchat C, Maungmingsook P, Rojanapanthu P, Gritsanaphan W. Topical application of *Garcinia mangostana* L. pericarp gel as an adjunct to periodontal treatment. *Complement Ther Med*. 2008;16(5):262–267. doi:10.1016/j.ctim.2007.12.004
12. Torrungruang K, Vichienroj P, Chutimaworapan S. Antibacterial activity of mangosteen pericarp extract against cariogenic *Streptococcus mutans*. *CU Dent J*. 2007;30:1–10. https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.908.2175&rep=rep1&type=pdf. Accessed June 18, 2021.
13. Hrishi TS, Menon SK, Kundapur PP, Gupta S. Comparative evaluation of GCF total antioxidant capacity in chronic periodontitis patients before and after non-surgical periodontal therapy. *Int J Dent Res Dev*. 2016;6(6):11–16. https://www.academia.edu/30971941/COMPARATIVE_EVALUATION_OF_GCF_TOTAL_ANTIOXIDANT_CAPACITY_IN_CHRONIC_PERIODONTITIS_PATIENTS_BEFORE_AND_AFTER_NON_SURGICAL_PERIODONTAL_THERAPY. Accessed June 18, 2021.
14. Rahman S, Gayathri GV, Mehta DS. A clinico-microbiological and biochemical study evaluating the adjunctive use of antimicrobial photodynamic therapy and local drug delivery of 1.2% simvastatin gel compared to scaling and root planing alone. *Photodiagnosis Photodyn Ther*. 2020;32:102017. doi:10.1016/j.pdpdt.2020.102017
15. Chow SC, Shao J, Wang H. *Sample Size Calculations in Clinical Research*. 2nd ed. New York, NY: Chapman & Hall/CRC; 2008:71.
16. Macgregor ID. Comparison of the Silness–Loe (1964) index with gravimetric measurement of dental plaque. *Clin Prev Dent*. 1987;9(6):9–12. PMID:3505836.
17. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J*. 1975;25(4):229–235. PMID:1058834.
18. Chapple IL, Brock G, Eftimiadi C, Matthews JB. Glutathione in gingival crevicular fluid and its relation to local antioxidant capacity in periodontal health and disease. *Mol Pathol*. 2002;55(6):367–373. doi:10.1136/mp.55.6.367
19. Rams TE, Slots J. Local delivery of antimicrobial agents in the periodontal pocket. *Periodontol 2000*. 1996;10:139–159. doi:10.1111/j.1600-0757.1996.tb00072.x

20. Mombelli A, Samaranayake LP. Topical and systemic antibiotics in the management of periodontal diseases. *Int Dent J*. 2004;54(1):3–14. doi:10.1111/j.1875-595x.2004.tb00246.x
21. Bollen CM, Quirynen M. Microbiological response to mechanical treatment in combination with adjunctive therapy. A review of the literature. *J Periodontol*. 1996;67(11):1143–1158. doi:10.1902/jop.1996.67.11.1143
22. Francetti L, Trombelli L, Lambordo G, et al. Evaluation of efficacy of enamel matrix derivative in the treatment of infrabony defects: A 24-month multicentre study. *Int J Periodontics Restorative Dent*. 2005;25(5):461–473. doi:10.11607/prd.00.0652
23. Pedraza-Chaverri J, Cárdenas-Rodríguez N, Orozco-Ibarra M, Pérez Rojas JM. Medicinal properties of mangosteen (*Garcinia mangostana*). *Food Chem Toxicol*. 2008;46(10):3227–3239. doi:10.1016/j.fct.2008.07.024
24. Sagar S. Role of natural toothbrushes in containing oral microbial flora – a review. *Asian J Pharm Clin Res*. 2015;8(4):29–33. https://innovareacademics.in/journals/index.php/ajpcr/article/view/6558. Accessed June 18, 2021.
25. Iinuma M, Tosa H, Tanaka T, et al. Antibacterial activity of xanthones from guttiferaeous plants against methicillin-resistant *Staphylococcus aureus*. *J Pharm Pharmacol*. 1996;48(8):861–865. doi:10.1111/j.2042-7158.1996.tb03988.x
26. Sundaram BM, Gopalakrishnan C, Subramanian S, Shankaranarayanan D, Kameswaran L. Antimicrobial activities of *Garcinia mangostana*. *Planta Med*. 1983;48(1):59–60. doi:10.1055/s-2007-969882
27. Samprasit W, Opanasopit P, Sukma M, Kaomongkolgit R. Antibacterial activity of *Garcinia mangostana* extracts on oral pathogens. *Minerva Stomatol*. 2014;63(7–8):249–257. PMID:25299359.
28. Vinayaka AM, Gayathri GV, Triveni MG. Comparative evaluation of the efficacy of 4% mangosteen gel and 1% chlorhexidine digluconate gel in management of patients with chronic gingivitis: A randomized clinical trial. *Int J Res Ayurveda Pharm*. 2021;12(6):6–11. doi:10.7897/2277-4343.1206161
29. Chomnawang MT, Surassmo S, Nukoolkarn VS, Gritsanapan W. Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. *J Ethnopharmacol*. 2005;101(1–3):330–333. doi:10.1016/j.jep.2005.04.038
30. Panchinda W. A follow up study of chronic ulcer healing dressing with 1.5 percent mangostin cream [M.Sc. thesis]. Bangkok, Thailand: Mahidol University; 1992.
31. Kusuma K. A comparison study of the foot ulcer healing between dressing with mangostin cream and normal saline wet dressing in diabetic patient [MSN thesis]. Bangkok, Thailand: Mahidol University; 2003.
32. Kaomongkolgit R, Jamdee K. Inhibitory effect of alpha-mangostin on adhesion of *Candida albicans* to denture acrylic. *Open Dent J*. 2015;9:388–392. doi:10.2174/1874210601509010388
33. Vemanaradhya GG, Emani S, Mehta DS, Bhandari S. Effect of 1.2% of simvastatin gel as a local drug delivery system on gingival crevicular fluid interleukin-6 & interleukin-8 levels in non surgical treatment of chronic periodontitis patients. *Arch Oral Bio*. 2017;82:55–61. doi:10.1016/j.archoralbio.2017.05.022
34. Zarena AS, Sankar KU. A study of antioxidant properties from *Garcinia mangostana* L. pericarp extract. *Acta Sci Pol Technol Aliment*. 2009;8(1):23–34. https://www.food.actapol.net/pub/3_1_2009.pdf. Accessed June 18, 2021.

Comparison of two techniques in gingival recession treatment: A randomized one-year clinical follow-up study

Mustafa Serdar Evginer[1,B–D,F], Ebru Olgun[1,A,C,E,F], Hanife Merva Parlak[2,C,D,F], Anil Barak Dolgun[3,B,C,F], Huseyin Gencay Keceli[2,A–F]

[1] Department of Periodontology, Faculty of Dentistry, Kirikkale University, Turkey
[2] Department of Periodontology, Faculty of Dentistry, Hacettepe University, Ankara, Turkey
[3] College of Science, Engineering and Health, Royal Melbourne Institute of Technology (RMIT University), Melbourne, Australia

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):121–130

Address for correspondence
Huseyin Gencay Keceli
E-mai: monsieur_gencay@yahoo.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
The authors express their gratitude to the entire working staff for their extraordinary efforts during their co-working period. The study was presented at CED-IADR/NOF Oral Health Research Congress organized on September 20–23, 2017 in Vienna, Austria.

Received on March 21, 2021
Reviewed on May 13, 2021
Accepted on May 18, 2021

Published online on March 31, 2022

Abstract

Background. Gingival recession (GR) is highly prevalent in the general population and represents a significant concern for patients and clinicians. Various surgical techniques have been proposed to treat gingival recession. Well-designed trials with clinician- and patient-based parameters, evaluating the envelope connective tissue graft (E-CTG) and semilunar coronally advanced flap (SCAF) techniques are still needed.

Objectives. The aim of this trial was to compare the effectiveness of E-CTG and SCAF in the treatment of GR during a 1-year follow-up.

Material and methods. A total of 42 patients with GR were treated with E-CTG ($n = 20$) or SCAF ($n = 22$). Clinician-based recordings of recession depth (RD), recession width (RW), probing depth (PD), clinical attachment level (CAL), keratinized tissue width (KTW), tissue thickness (TT), clinical attachment gain (CAG), root coverage (RC), keratinized tissue change (KTC), and wound healing index (WHI), as well as patient-based parameters of dentine hypersensitivity (DH), tissue appearance, patient expectations, and esthetics were collected at baseline (BL), 6 weeks (T_1), 6 months (T_2), and 1 year (T_3).

Results. After the treatment, E-CTG demonstrated better outcomes than SCAF in terms of CAG (50.70% vs. 33.33%), RC (85.60% vs. 35.60%) and KTC (1.70 ±1.49 mm vs. 0.36 ±0.96 mm) at T_3. Similar findings were detected in terms of WHI, tissue appearance, patient expectations, and esthetics. Although unpleasant surgical experience was recorded, better results were obtained after E-CTG in terms of DH and meeting the RC expectations.

Conclusions. Despite it being more uncomfortable surgical experience and the risk of keloid formation, E-CTG was superior to SCAF in terms of RC percentage, reducing DH and obtaining satisfactory RC. However, it is still necessary to improve patient comfort in the case of E-CTG.

Keywords: gingival recession, connective tissue, treatment outcomes, esthetics, patient-reported outcomes

Cite as
Evginer MS, Olgun E, Parlak HM, Dolgun AB, Keceli HG. Comparison of two techniques in gingival recession treatment: A randomized one-year clinical follow-up study. *Dent Med Probl.* 2022;59(1):121–130. doi:10.17219/dmp/137621

DOI
10.17219/dmp/137621

Introduction

A patient is considered a candidate for surgical gingival recession (GR) treatment when at least one of the following criteria is met: persistent gingival inflammation; progressive GR; or progressive attachment loss, following phase I periodontal therapy, or in case of planned orthodontic/restorative interventions.[1] In such cases, after the decision-making process, GR can be treated with various surgical techniques, including pedicle flaps, soft tissue grafts, guided tissue regeneration, and tissue substitutes, and their combinations.[2,3]

Connective tissue graft (CTG) is a predictable method with promising esthetic outcomes in GR treatment and is defined as the gold standard protocol for this purpose.[3,4] From among its overlying flap modifications, the envelope technique (E-CTG) consists in 2 horizontal incisions and the elevation of the split-thickness flap by undermining from the apical portion of GR. Envelope CTG is preferred in the treatment of shallow GR owing to its high gingival margin (GM) stability and less flap advancement needed.[5] This technique also has a high clinical success rate varying from 88.3% to 96.8% of root coverage (RC).[6–8] Besides its advantages, including the lack of vertical incisions and the preservation of the adjacent papillae, technical difficulties and the surgical impact on 2 different regions still make clinicians explore further alternatives.

From among the RC techniques, semilunar coronally advanced flap (SCAF), described by Tarnow,[9] consists in positioning the flap coronally by means of sulcular and horizontal apical incisions without disturbing the integration of the adjacent papillae. Moreover, the vestibule depth and the color match of the tissues can be maintained.[10] The RC% success of SCAF exhibits a wide range (41.8–90.1%).[6,7,11,12] Bittencourt et al. compared SCAF with E-CTG and both modalities exhibited successful RC% (89.3% vs. 96.3%), while E-CTG represented better patient-based outcomes in the treatment of GR as compared to SCAF.[7] Although SCAF is a practical procedure with a similar indication as in the case of E-CTG, only one study group compared the clinical outcomes obtained with SCAF and E-CTG in 2 relevant papers.[6,7] Taking this into account, further well-designed trials evaluating these 2 techniques by using clinician- and patient-based parameters are still required. Thus, the present study aimed to compare the E-CTG and SCAF techniques in Miller Class I GR treatment.

Material and methods

Study design

The present single-center, parallel-group clinical study, registered at https://www.clinicaltrials.gov/ (NCT04109794), was designed as a prospective, comparative, randomized, and single-blinded study. It was conducted between December 2012 and May 2014 with the permission of the Institutional Review Board at Kirikkale University, Turkey (protocol No. 12/12-3 of November, 12, 2012).

Individuals and randomization

From among 120 patients, 42 patients (37 females and 5 males), aged 20–54 years, with single Miller Class I GR defects ≤3 mm at their upper anterior or premolar teeth were chosen by considering the following inclusion criteria: systemically healthy patients; age >18 years; identifiable cemento-enamel junction (CEJ); and probing depth (PD) ≤3 mm. The exclusion criteria were as follows: periodontal surgery experience in the past 2 years; excessive contacts, mobility, caries, or a restoration in the relevant tooth; the loss of tooth vitality; smoking; and pregnancy. Written informed consent forms were completed by the individuals and necessary treatment was provided according to the current standards of health care. One investigator randomly assigned the patients, with a 1:1 allocation ratio, to the E-CTG and SCAF groups by using simple randomization without blocking (a computer-generated randomization scheme) (Fig. 1).

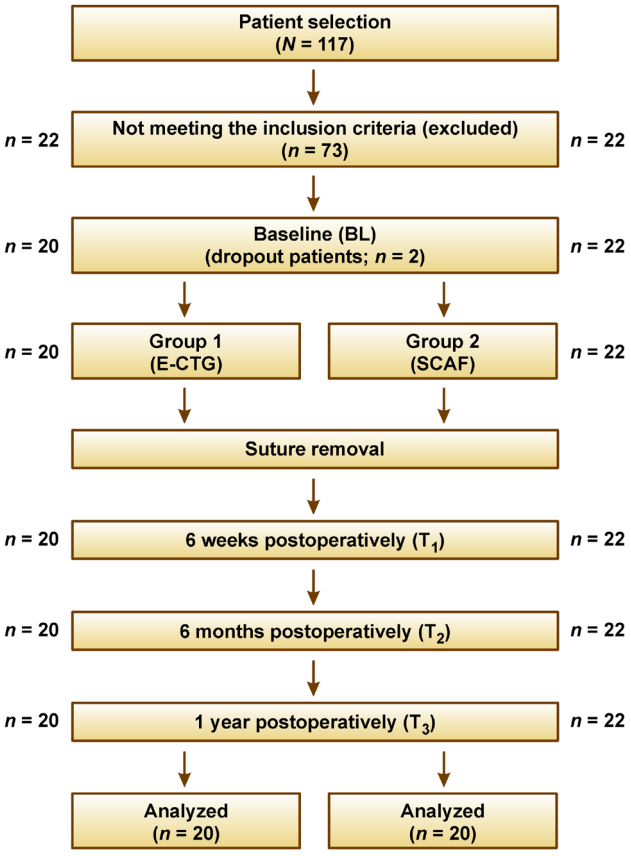

Fig. 1. Flow chart of the study

E-CTG – envelope connective tissue graft; SCAF – semilunar coronally advanced flap.

Allocation concealment and calibration

Number-labeled opaque envelopes that contained the name of the assigned method were used for concealing the allocation. A 90.7% (a coefficient value of 0.91) calibration level was achieved after measuring the distance from CEJ to GM, i.e., recession depth (RD), 3 times.

Phase I therapy

Personalized oral hygiene instructions focusing on the proper tooth brushing techniques (the roll technique directing to the coronal aspect of GR) were provided. A rubber cup with a non-abrading paste was used for professional prophylaxis, and if necessary, occlusal adjustments and/or bite guards were completed and delivered. The patients were not transferred to the surgical phase of the study until they achieved adequate hygiene and gingival health (the full-mouth plaque and bleeding scores <15%).[13]

Clinician-based variables

Periodontal variables

Another author blinded to the type of intervention measured the clinical variables at the mid-buccal locations of the treated teeth at baseline (BL), 6 weeks (T_1), 6 months (T_2), and 1 year (T_3) following the interventions by using a Michigan-O periodontal probe (Hu-Friedy, Chicago, USA), and the measurements were justified to the nearest 0.1 mm with a caliper (Kohdent Roland Kohler Medizintechnik, Stockach, Germany). The following variables were evaluated: gingival index (GI)[14]; plaque index (PI)[15]; RD as the distance between CEJ and GM; recession width (RW) as the horizontal distance between 2 recession borders at the CEJ level; PD as the distance between GM and the base of the gingival sulcus; clinical attachment level (CAL) as the distance between CEJ and the base of the gingival sulcus; and keratinized tissue width (KTW) as the distance between GM and the mucogingival junction (MGJ). Tissue thickness (TT) was measured after sticking a spreader (Technical & General Ltd., London, UK) into the gingiva 1.5 mm below GM, and then adjusting its silicon stopper.[16] Changes in the variables of RD, CAL and KTW were calculated to determine the clinical attachment gain (CAG), RC and keratinized tissue change (KTC) values. The number and proportion of defects, showing complete RC (CRC) were also calculated. Recession depth was the primary outcome measure, while the secondary variables included CAL, KTW and TT.

Wound healing index (WHI)[17]

The wound healing index was recorded 2 weeks after the surgery. The wound surface was visually inspected and the soft tissue healing was defined as 'uneventful' (score 1), 'slightly disturbed' (score 2) or 'poor' (score 3) based on the presence and severity of the following items: patient discomfort; erythema; edema; suppuration; and flap dehiscence.

Patient-based variables

Dentine hypersensitivity (DH)[18]

The evaporative air stimulus method was utilized at BL and T_2. After the placement of finger(s) for protecting the nearby teeth, the GR sites were subjected to an evaporative stimulus, which comprised a 1-second air blast from a distance of 1–3 mm by using an air spray of a pressure of 40–65 psi and a temperature of 19 ±5°C. After application, the individuals were requested to give a score of their DH between 0 (no pain) and 10 (extreme pain).

Tissue appearance[19]

The patients were asked to score the consistency, contour, color match, keloid formation degree, and contiguity of their treated sites at T_2. The scores were collected as points shown in parentheses. Consistency was assessed as firm (1 pt) or spongy (0 pt); contour as the presence (2 pt) or absence (0 pt) of knife-edged and scalloped GM; color match as excellent (3 pt), good (2 pt), adequate (1 pt), or unsatisfactory (0 pt); keloid formation degree as absent (1 pt) or present (0 pt); and contiguity as the presence (–1 pt) or absence (0 pt) of each perceptible incision mark.

Patient expectations[19]

The patients were requested to rate their treatment results at T_2 according to their expectations as satisfactory or not in terms of appearance, experience and the obtained RC.

Esthetics[19]

The level of esthetics was evaluated as excellent, good, fair, or poor.

Surgical interventions

The interventions were performed by one of the researchers, and are shown in Fig. 2 and Fig. 3. Before the surgery, a chlorhexidine gluconate mouth rinse (0.2%) (Klorhex®; Drogsan İlaçları, Ankara, Turkey) was given for intra-oral antisepsis. Articaine hydrochloride with epinephrine (1:100,000) (Ultracaine® D-S forte; Hoechst Marion Roussel, Frankfurt, Germany) was used to obtain anesthesia at the surgical region. Root planing was performed with hand instruments (Gracey curettes; Hu-Friedy), and if necessary, root convexity was eliminated by using fine-grain finishing burs (Hager & Meisinger, Düsseldorf, Germany). After planing and shaping, the surface was irrigated with sterile saline.

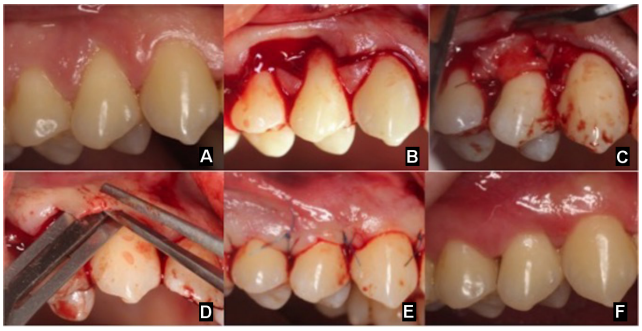

Fig. 2. Surgical interventions in the envelope connective tissue graft (E-CTG) group

A – preoperative clinical view; B – envelope incisions; C – flap elevation and CTG fixation; D – release of flap tension; E –wound closure; F – 1-year follow-up.

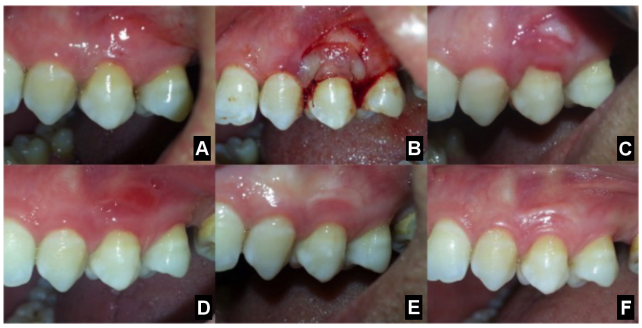

Fig. 3. Surgical interventions in the semilunar coronally advanced flap (SCAF) group

A – preoperative clinical view; B – postoperative clinical view; C – 10-day follow-up; D – 6-week follow-up; E – 6-month follow-up; F – 1-year follow-up.

E-CTG group[6]

Horizontal incisions were made from the base of the adjacent papilla triangles and connected with a sulcular incision extending to the MGJ level by uniformly undermining as split-thickness elevation. Connective tissue graft was taken from the premolar-molar region of the lateral palate with the use of the single-incision technique,[20] placed under the prepared envelope flap, secured with sling sutures (Ruschmed; İpek Plastik, Istanbul, Turkey), and covered by the positioning flap margin at CEJ with proximal sutures (Dogsan Tibbi Malzeme San, Trabzon, Turkey). The donor site was closed with continuous sutures (Dogsan Tibbi Malzeme San) (Fig. 2).

SCAF group[9]

A mesiodistally directed, curved incision, following the GM outline was made from the apical part of MGJ, sufficiently apical to place the apical end of the flap onto the alveolar bone, and was finished at least 2 mm under the tips of the adjacent papillae. The 2nd incision was started as a sulcular incision, continued as split-thickness dissection and finished by connecting it to the 1st incision. Then, the coronal margin of the elevated tissue was positioned at CEJ by gently sliding with wet gauze pressure. Care was taken to create an adequate amount of flap height in order to preserve blood supply. The flap was secured to the cervical region of the tooth with a sling suture (Ruschmed; İpek Plastik) for additional stabilization (Fig. 3).

Postsurgical period

For reducing pain and edema, extra-oral cold compress and analgesic anti-inflammatory medicines (Ibuprofen 100 mg; Sanovel Ilac, Istanbul, Turkey) were given. Oral hygiene activities were paused for 4 weeks, and a mouth rinse (0.2% chlorhexidine gluconate, Klorhex; Drogsan İlaçları) was prescribed instead. The patients were warned to watch out for traction or excessive trauma. The sutures were removed 2 weeks after the surgery, and the patients had recall appointments consisting of professional plaque control and hygiene instructions once a month.

Sample size calculation and statistical analysis

The sample size was determined by means of the NCSS PASS software, v. 11.0.8, assuming $\alpha = 0.05$, with the two-sided t test. Accordingly, 22 patients per group achieved a power of 81% to detect a difference of 0.6 ±0.7 mm in RD.[12]

The IBM SPSS Statistics for Windows software, v. 21.0 (IBM Corp., Armonk, USA), was used for statistical analysis and the significance level was set at $p < 0.05$. Quantitative variables were expressed as the mean and standard deviation ($M \pm SD$), and median and minimum–maximum (Me (min–max)) values. For nominal data, frequency and percentage (n (%)) were provided. The Shapiro–Wilk test was used to check the normality assumption. Nonparametric statistical tests were used, as the data did not conform to normal distribution. The intergroup comparisons of quantitative variables were made using the Mann–Whitney U test, whereas the Friedman test was used for repeated measures analysis. The distribution of qualitative variables among the study groups was analyzed with the Fisher–Freeman–Halton test and the χ^2 test.

To detect a difference of 0.6 mm with regard to the null hypothesis, the group sample sizes of 20 and 22 achieved a post hoc power of 59%. With a 0.05 significance level, using the two-sided two-sample t test and the Mann–Whitney U test, and assuming that the actual distribution was uniform, the mean difference in both groups was 1.3 mm; the alternative hypothesis was that the mean difference in the SCAF group would be 0.8 mm with the SD values of 0.9 mm and 0.8 mm for the E-CTG and SCAF groups, respectively.

Results

A satisfactory level of plaque control and satisfactory bleeding scores were obtained after phase I therapy. Two individuals from the E-CTG group did not attend

Table 1. Demographic information and the distribution of the recession sites

Variable		E-CTG (n = 20)	SCAF (n = 22)	Total (N = 42)	p-value
Age [years]	*M* ±*SD*	35.00 ±10.45	37.32 ±9.99	36.21 ±10.15	0.384
	Me (min–max)	34.0 (20–54)	35.5 (20–54)	34.5 (20–54)	
Gender *n* (%)	male	4 (20.0)	1 (4.5)	5 (11.9)	0.174
	female	16 (80.0)	21 (95.5)	37 (88.1)	
Tooth *n* (%)	incisor	4 (20.0)	3 (13.6)	7 (16.7)	0.692
	canine	2 (10.0)	6 (27.3)	8 (19.0)	
	premolar	14 (70.0)	13 (59.1)	27 (64.3)	

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

the intervention stage and the study was completed with 42 patients (Fig. 1). The trial ended after 1 year of patient follow-up. Demographics and GR site distribution (7 incisors, 8 canines and 27 premolars) are presented in Table 1. The mean age of the individuals was 36.21 ±10.15 years, and no intergroup difference was detected in terms of age or gender.

Clinician-based variables (Tables 2, 3)

Gingival index (GI)

In the beginning, the mean GI value was almost the same for both groups ($p > 0.05$) and after the surgery, a slight reduction was noticed. During the follow-up,

Table 2. Clinician-based variables

Variable	Time of assessment	E-CTG (n = 20)		SCAF (n = 22)		p-value
		M ±*SD*	*Me* (min–max)	*M* ±*SD*	*Me* (min–max)	
GI	BL	0.55 ±0.61	0.5 (0–2.0)	0.55 ±0.67	0 (0–2.0)	0.887
	T_1	0.35 ±0.49	0 (0–1.0)	0.43 ±0.50	0 (0–1.0)	0.567
	T_2	0.50 ±0.69	0 (0–2.0)	0.18 ±0.39	0 (0–1.0)	0.097
	T_3	0.40 ±0.50	0 (0–1.0)	0.09 ±0.29*	0 (0–1.0)	0.020‡
PI	BL	0.40 ±0.75	0 (0–3.0)	0.45 ±0.60	0 (0–2.0)	0.509
	T_1	0.15 ±0.37	0 (0–1.0)	0.36 ±0.49	0 (0–1.0)	0.120
	T_2	0.45 ±0.69	0 (0–2.0)	0.23 ±0.43	0 (0–1.0)	0.307
	T_3	0.45 ±0.60	0 (0–2.0)	0.14 ±0.35	0 (0–1.0)	0.050
RD [mm]	BL	1.54 ±0.91	1.8 (0.1–3.0)	1.58 ±0.96	1.5 (0.1–4.0)	0.948
	T_1	0.50 ±0.88**	0.3 (0–4.0)	1.33 ±1.00	1.3 (0–3.5)	0.001‡
	T_2	0.44 ±0.68**	0.3 (0–3.0)	1.07 ±0.92*	1.0 (0–3.5)	0.009‡
	T_3	0.21 ±0.48**	0 (0–2.0)	0.82 ±0.97**,†	0.5 (0–3.0)	0.074
RW [mm]	BL	2.89 ±1.43	3 (0.4–6.0)	2.89 ±1.69	3.0 (0.3–6.0)	0.758
	T_1	0.77 ±0.81**	0.6 (0–3.0)	1.96 ±1.48*	2.0 (0–5.0)	0.004‡
	T_2	0.72 ±0.78**	0.5 (0–2.5)	1.64 ±1.37*	1.5 (0–4.5)	0.027‡
	T_3	0.32 ±0.52**	0 (0–1.7)	1.11 ±1.29**	1.0 (0–4.0)	0.090
PD [mm]	BL	1.40 ±0.60	1.0 (1.0–3.0)	1.31 ±0.48	1.0 (1.0–2.0)	0.747
	T_1	1.20 ±0.41	1.0 (1.0–2.0)	1.22 ±0.43	1.0 (1.0–2.0)	0.832
	T_2	1.15 ±0.37	1.0 (1.0–2.0)	1.14 ±0.35	1.0 (1.0–2.0)	0.901
	T_3	1.05 ±0.22	1.0 (1.0–2.0)	1.13 ±0.35	1.0 (1.0–2.0)	0.347
CAL [mm]	BL	2.94 ±1.13	3.0 (1.1–5.0)	2.90 ±1.19	3.0 (1.1–6.0)	0.719
	T_1	1.70 ±1.10	1.5 (1.0–6.0)	2.56 ±1.15	2.5 (1.0–5.5)	0.004‡
	T_2	1.59 ±0.96*	1.3 (1.0–5.0)	2.20 ±1.09	2.0 (1.0–5.5)	0.021‡
	T_3	1.26 ±0.68**,†	1.0 (1.0–4.0)	1.95 ±1.01	1.5 (1.0–4.0)	0.042‡
KTW [mm]	BL	3.35 ±1.93	2.3 (1.5–8.0)	4.27 ±1.02	4.0 (2.0–7.0)	0.021‡
	T_1	4.93 ±1.56*	5.0 (3.0–9.0)	4.34 ±1.13	4.0 (2.5–7.0)	0.255
	T_2	4.95 ±1.36*	5.0 (3.0–8.0)	4.55 ±1.14	5.0 (2.0–6.0)	0.414
	T_3	5.05 ±1.23**	5.0 (3.0–8.0)	4.64 ±1.09	5.0 (2.0–6.0)	0.327
TT [mm]	BL	1.08 ±0.37	1.0 (0.5–1.5)	1.07 ±0.44	1.0 (0.5–2.0)	0.776
	T_1	1.83 ±0.29**	2.0 (1.0–2.0)	1.45 ±0.41*	1.5 (1.0–2.5)	0.001‡
	T_2	1.83 ±0.37**	2.0 (1.0–2.5)	1.52 ±0.39*	1.5 (1.0–2.0)	0.021‡
	T_3	1.88 ±0.39**	2.0 (1.5–2.5)	1.59 ±0.40**	1.5 (1.0–2.0)	0.054
WHI	2 weeks	1.65 ±0.67	2.0 (1.0–3.0)	1.95 ±0.89	2.0 (1.0–3.0)	0.280

GI – gingival index; PI – plaque index; RD – recession depth; RW – recession width; PD – probing depth; CAL – clinical attachment level; KTW – keratinized tissue width; TT – tissue thickness; WHI – wound healing index; BL – baseline; T_1 – 6 weeks postoperatively; T_2 – 6 months postoperatively; T_3 – 1 year postoperatively; * significantly different as compared to BL ($p < 0.05$); ** significantly different as compared to BL ($p < 0.001$); † significantly different as compared to T_1 ($p < 0.05$); ‡ significantly different between the groups.

Table 3. Clinical attachment gain (CAG), root coverage (RC) and keratinized tissue change (KTC) values

Variable	Time of assessment	E-CTG (n = 20)				SCAF (n = 22)				p-value	p-value (%)
		M ±*SD*	*Me* (min–max)	*M* ±*SD* (%)	*Me* (min–max) (%)	*M* ±*SD*	*Me* (min–max)	*M* ±*SD* (%)	*Me* (min–max) (%)		
CAG [mm]	T_1	1.19 ±1.30	1.05 (−3.0–2.8)	37.10 ±36.53	50.00 (−100.0–70.0)	0.34 ±0.80	0.50 (−2.0–2.0)	7.99 ±32.59	12.50 (−100.0–66.7)	0.001‡	<0.001‡
	T_2	1.30 ±1.31	1.05 (−2.0–3.5)	39.10 ±35.21	50.00 (−66.7–75.0)	0.69 ±0.97	0.50 (−1.5–2.5)	18.30 ±34.30	20.83 (−75.0–71.4)	0.065	0.020‡
	T_3	1.63 ±1.17	1.75 (−1.0–3.5)	50.70 ±28.01	57.50 (−33.3–75.0)	0.94 ±1.10	1.00 (−1.5–2.5)	26.41 ±39.36	33.33 (−75.0–71.4)	0.064	0.014‡
RC [mm]	T_1	1.04 ±1.07	1.00 (−2.0–2.8)	73.30 ±45.14	85.00 (−100.0–100.0)	0.25 ±0.75	0.50 (−2.0–2.0)	8.22 ±66.97	22.50 (−200.0–100.0)	0.001‡	<0.001‡
	T_2	1.10 ±0.93	1.00 (−1.0–2.9)	72.00 ±38.42	80.00 (−50.0–100.0)	0.50 ±0.77	0.50 (−1.5–2.0)	11.78 ±88.60	25.00 (−300.0–100.0)	0.030‡	0.001‡
	T_3	1.33 ±0.86	1.25 (0–2.9)	85.60 ±30.47	100.00 (0–100.0)	0.76 ±0.82	1.00 (−1.5–2.0)	35.60 ±113.91	77.50 (−400.0–100.0)	0.060	0.111
KTC [mm]	T_1	1.57 ±1.19	1.25 (1.0–4.0)	71.80 ±67.53	50.00 (16.7–233.3)	0.07 ±0.80	0 (−1.0–1.5)	3.13 ±19.37	0 (−33.3–33.3)	<0.001‡	<0.001‡
	T_2	1.60 ±1.30	1.25 (1.0–4.0)	75.10 ±71.27	55.00 (16.7–233.3)	0.27 ±0.99	0.50 (−2.0–2.0)	8.31 ±22.85	10.00 (−40.0–50.0)	0.002‡	<0.001‡
	T_3	1.70 ±1.49	1.75 (1.0–4.5)	82.70 ±82.14	63.30 (16.7–300.0)	0.36 ±0.96	0.25 (−2.0–2.0)	10.56 ±22.38	5.55 (−40.0–50.0)	0.003‡	0.001‡
CRC *n* (%)	T_1	7 (35.00)				2 (9.09)				0.041‡	
	T_2	6 (30.00)				2 (9.09)				0.084	
	T_3	12 (60.00)				10 (45.45)				0.345	

CRC – complete root coverage; ‡ statistically significant.

the reduction of GI continued in the SCAF group, and reached a statistically significant intra- (p = 0.047) and intergroup difference (p = 0.020) at T_3, also with the effect of a slight but not statistically significant rise in the E-CTG patients.

Plaque index (PI)

At BL, the PI values were 0.40 ±0.75 and 0.45 ±0.60 for the E-CTG and SCAF groups, respectively (p > 0.05). After the surgery, although not significantly, PI showed a reduction until T_1, and then turned back to its BL level in the E-CTG group. In the SCAF group, it showed a regular but not statistically significant reduction tendency, and did not reach a statistically significant difference as compared to the E-CTG group.

Recession depth (RD) and recession width (RW)

At BL, the RD and RW values were similar for both groups (p > 0.05). Although SCAF showed its effect later (T_3), both treatment modalities provided a statistically significant RD and RW reduction (p < 0.001). According to the intergroup comparison, the mean postsurgical RD and RW values were lower in the E-CTG group, and the differences were statistically significant at T_1 and T_2 (p < 0.05).

Probing depth (PD)

Probing depth did not show any time-dependent or intergroup differences during the study period (p > 0.05).

Clinical attachment level (CAL)

While the CAL values for both groups were similar at BL (p > 0.05), the E-CTG group demonstrated a higher CAL reduction after the surgery and the difference was maintained until the end of the follow-up period (p < 0.05).

Keratinized tissue width (KTW)

In the beginning, the SCAF group exhibited a higher mean KTW value (p = 0.021). After the treatment, an increase in KTW was detected only in the E-CTG group (p < 0.05) and the intergroup comparison of the KTW values showed statistical similarity at all follow-up visits (p > 0.05).

Tissue thickness (TT)

At BL, the number of individuals having TT ≥ 1 mm was 16 for the E-CTG group and 17 for the SCAF group. The mean TT values were similar at BL (p > 0.05) and increased after both surgery types (p < 0.05). However,

the increase was higher in the E-CTG group, and reached a statistically significant difference as compared to the SCAF group at T_1 and T_2 ($p < 0.05$).

Clinical attachment gain (CAG)

The millimetric values for CAG demonstrated an intergroup difference only at T_1 ($p = 0.001$), whereas CAG% was significantly higher in the E-CTG group at all follow-up visits ($p < 0.05$). At T_3, CAG was 50.70% and 33.33% in the E-CTG and SCAF groups, respectively.

Root coverage (RC)

Higher RC was detected in the E-CTG group at T_1 ($p < 0.001$) and T_2 ($p < 0.05$). At T_3, the E-CTG and SCAF groups showed mean RC values of 1.33 ±0.86 (85.60%) and 0.76 ±0.82 (35.60%), respectively, but the difference was not statistically significant. While the difference in the number of defects, showing CRC reached statistical significance at T_1 ($p = 0.041$), the differences between the groups at T_2 ($p = 0.084$) and T_3 ($p = 0.345$) were not statistically significant. Complete RC was 12/20 (60.00%) and 10/22 (45.45%) for the E-CTG and SCAF groups, respectively.

Keratinized tissue change (KTC)

While the mean KTC was 1.70 ±1.49 mm in the E-CTG, it was calculated as 0.36 ±0.96 mm in the SCAF group at T_3. Keratinized tissue change was statistically significantly higher for E-CTG at all measurement times ($p < 0.05$).

Wound healing index (WHI)

The mean WHI values were 1.65 ±0.67 and 1.95 ±0.89 in the E-CTG and SCAF groups, respectively, and the intergroup difference was not statistically significant.

Patient-based variables (Table 4)

Dentin hypersensitivity (DH)

In the beginning, DH was similar for both groups. After the treatment, a significant reduction in DH was noted for both groups ($p < 0.001$), whereas the E-CTG group showed a higher decrease ($p = 0.008$), demonstrating an analogy with the RC outcomes.

Tissue appearance

Except for keloid formation degree, none of the parameters related to tissue appearance showed a remarkable intergroup difference ($p > 0.05$). According to the analysis, the SCAF group revealed more keloid formation as compared to the E-CTG group ($p = 0.028$).

Patient expectations

Regarding their comments about the treatment results, the patients treated with SCAF perceived their surgical experience as better ($p = 0.006$), whereas the E-CTG patients were happier with their obtained RC outcomes ($p = 0.012$). However, both modalities created similar comments about tissue appearance ($p > 0.05$).

Table 4. Patient-based variables

Variable		Time of assessment	E-CTG ($n = 20$)	SCAF ($n = 22$)	Total ($N = 42$)	*p*-value
DH *M ±SD*		BL	6.20 ±1.79	5.54 ±1.59	5.85 ±1.70	0.133
		T_2	0.30 ±1.12	2.50 ±3.37	1.45 ±2.76	0.008‡
p-value			<0.001	<0.001	<0.001	–
Tissue appearance *M ±SD*	consistency	T_2	0.75 ±0.44	0.50 ±0.52	0.62 ±0.49	0.100
	contour		0.70 ±0.97	1.09 ±1.01	0.90 ±1.00	0.209
	color match		1.20 ±0.89	1.22 ±1.02	1.21 ±0.95	0.979
	keloid formation degree		0.75 ±0.44	0.40 ±0.50	0.57 ±0.50	0.028‡
	contiguity		–0.55 ±0.51	–0.45 ±0.51	–0.50 ±0.51	0.542
Patient expectations *M ±SD*	appearance	T_2	0.75 ±0.44	0.50 ±0.51	0.62 ±0.49	0.100
	experience		0.30 ±0.47	0.72 ±0.45	0.52 ±0.51	0.006‡
	obtained RC		0.90 ±0.30	0.54 ±0.51	0.71 ±0.46	0.012‡
Esthetics *n* (%)	excellent	T_2	15 (75.0)	13 (59.1)	28 (66.7)	0.500
	good		5 (25.0)	6 (27.3)	11 (26.2)	
	fair		0	2 (9.1)	2 (4.8)	
	poor		0	1 (4.5)	1 (2.4)	

DH – dentin hypersensitivity; ‡ statistically significant.

Esthetics

At T_2, no difference was detected regarding the patient-based esthetic evaluation ($p > 0.05$) and the esthetics values were predominantly assembled around the 'excellent' (66.7%) and 'good' (26.2%) levels.

Discussion

The study aimed to compare E-CTG and SCAF in Miller Class I GR treatment, and both modalities showed successful clinical outcomes. While E-CTG provided better clinician-based results with regard to CAG, RC and KTC, and patient-based results in terms of DH and keloid formation, SCAF caused less postsurgical discomfort. In the present study, the E-CTG group had a mean RC of 85.60% and this value is consistent with the results of the clinical studies regarding E-CTG (88.3–96.8%).[6,7,8] On the other hand, the SCAF group resulted in 35.60 RC% at 1 year. This result is slightly inferior to the lower limit of the RC% range (41.8–90.1%) in the randomized clinical trials (RCTs) which reported the short-term success rate of SCAF by comparing it with coronally advanced flap (CAF), CTG or SCAF + ethylenediaminetetraacetic acid (EDTA).[6,7,11,12] The relevant literature and the present results revealed that SCAF was a less predictable RC method in comparison with E-CTG.

At BL, in spite of randomization, the SCAF group showed a higher mean KTW as compared to E-CTG and this should be considered a limitation that might have masked the actual effects of the techniques. At T_3, E-CTG provided greater KTC and this result seems conceivable due to the well-known clinical influence of CTG on a KTC increase, associated with the biological concept of the characteristics of the surface epithelium determined by the information residing in the connective tissue.[21] The present results showing a tendency for higher TT enhancement in the E-CTG patients also seem to be related to this phenomenon.

An increase in TT after CTG has been reported by various authors,[22,23] and also confirmed by the present evaluations. Although TT is a valuable determinant for GR development and CRC, the possible occurrence of excessive marginal thickening and the loss of the scalloped form should not be overlooked. In the present study, the lower scores given by the patients from the E-CTG group to their GM morphology seemed to arise from these circumstances. Aichelmann-Reidy et al., who used the same subjective tissue contour evaluation after CTG and the utilization of acellular dermal matrices, also reported similar lower values in their CTG-treated patients, possibly due to the same phenomenon.[19]

In the present study, the patients' perception of tissue appearance (consistency, contour, color match, and contiguity) as well as esthetics were similar for both groups. This result can be attributed to the meticulously applied surgical protocols and the absence of the epithelialized graft which could result in unacceptable tissue contour and esthetics. On the other hand, DH was more effectively reduced by E-CTG, with more satisfactory RC and less keloid formation. In GR studies, the main reducing factor for DH is the success rate of RC, and the higher DH reduction in the E-CTG group in the present trial seems to be related to the higher CRC%. In 2013, Douglas de Oliveira et al. surveyed the literature on the efficacy of RC techniques at reducing DH and according to the analysis, a definitive conclusion could not be made due to the inadequate number of well-conducted clinical trials; 9 articles reported DH decreases between 55.6 and 100.0, and presented DH reduction rates (95.2 and 54.9 for the E-CTG and SCAF groups, respectively) that were within this range.[24] Contrarily, the SCAF group reported a more comfortable therapeutic course as compared to the E-CTG group in terms of surgical experience, possibly due to the absence of a second surgical site and the shorter duration of SCAF.

Soft tissue handling that consists of flap design, vertical incisions, split/full-thickness elevation, flap tension, and coronal positioning is the critical factor affecting clinical outcomes.[25] There are significant differences between the 2 techniques, and thus the study could not be designed in a controlled fashion. Envelope CTG is advantageous in terms of absence of mucosal incisions and less interrupted vascularization at the apical region, while SCAF provides less flap tension and more coronal positioning. Therefore, higher RC might be anticipated in the case of E-CTG, whereas keloid formation would be more probable after SCAF. At the end of the present trial, the clinician- and patient-based variables indicated outcomes parallel with these predictions.

To date, only a few studies have evaluated the patient-based outcomes following RC procedures as an additional outcome.[22] Data is heterogeneous and its amount is still insufficient to ascertain the truth about the correspondence of these procedures to patient expectations by performing a meta-analysis.[3,26] One of the powerful aspects of this study is the presence of such parameters, including DH, tissue appearance, patient expectations, and the esthetic evaluation. The main clinical indication for the E-CTG and SCAF techniques usually resides within the RD range of 1–3 mm,[5] and this range was considered as an inclusion criterion in the present study. However, it is difficult to achieve sufficient power for detecting statistical significance within this range. Even though a caliper that makes the measurements to the nearest 0.1 mm was used to overcome this limitation, the post hoc sample size should reach a power of 59% to detect a significant difference. Moreover, most of GR studies involve the criterion of RD ≥ 2 mm owing to the probability of spontaneous RC during the follow-up.[27] Therefore, although the adopted RD range did not affect intergroup comparisons, it may

be considered a limitation of the trial in combination with the effect of a relatively small sample size, and deserves attention while comparing the results of the present study with the relevant literature.

Conclusions

Every patient desires the most successful result with the easiest therapeutic technique. Therefore, the question of 'Can SCAF be the more comfortable alternative of CTG in the treatment of GR to obtain the same satisfactory outcome?' is justified. Although both techniques were similarly effective in meeting the esthetic expectations of the patients, the results of the present study suggest that the reality that 'better RC outcomes are accompanied by comparably worse surgical experience' could not satisfy the above equation. Within the limitations of this study, the following conclusions can be made:

– E-CTG is more predictable in shallow Miller Class I GR treatment;
– although SCAF has a more comfortable therapeutic course, it is not recommended due to its low RC values;
– although E-CTG showed better results in terms of DH and meeting the RC expectations, patient comfort still needs to be improved; and
– further clinical studies comparing E-CTG and other surgical techniques are needed to support the present findings.

Trial registration

The present study was registered at https://www.clinicaltrials.gov/ under the number NCT04109794.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board at Kirikkale University, Turkey (protocol No. 12/12-3 of November, 12, 2012). All participants provided written informed consent prior to the investigations.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Mustafa Serdar Evginer https://orcid.org/0000-0002-4377-5143
Ebru Olgun https://orcid.org/0000-0001-7298-8589
Hanife Merva Parlak https://orcid.org/0000-0001-6508-9016
Anil Barak Dolgun https://orcid.org/0000-0002-2693-0666
Huseyin Gencay Keceli https://orcid.org/0000-0001-6695-2133

References

1. Merijohn GK. Management and prevention of gingival recession. *Periodontol 2000*. 2016;71(1):228–242. doi:10.1111/prd.12115
2. Keceli HG, Sengun D, Berberoğlu A, Karabulut E. Use of platelet gel with connective tissue grafts for root coverage: A randomized-controlled trial. *J Clin Periodontol*. 2008;35(3):255–262. doi:10.1111/j.1600-051X.2007.01181.x
3. Cairo F, Nieri M, Pagliaro U. Efficacy of periodontal plastic surgery procedures in the treatment of localized facial gingival recessions. A systematic review. *J Clin Periodontol*. 2014;41(Suppl 15):S44–S62. doi:10.1111/jcpe.12182
4. Chambrone L, Tatakis DN. Periodontal soft tissue root coverage procedures: A systematic review from the AAP Regeneration Workshop. *J Periodontol*. 2015;86(2 Suppl):S8–S51. doi:10.1902/jop.2015.130674
5. Allen AL. Use of the supraperiosteal envelope in soft tissue grafting for root coverage. I. Rationale and technique. *Int J Periodontics Restorative Dent*. 1994;14(3):216–227. PMID:7995692.
6. Bittencourt S, Del Peloso Ribeiro E, Sallum EA, Sallum AW, Nociti FH Jr., Casati MZ. Comparative 6-month clinical study of a semilunar coronally positioned flap and subepithelial connective tissue graft for the treatment of gingival recession. *J Periodontol*. 2006;77(2):174–181. doi:10.1902/jop.2006.050114
7. Bittencourt S, Del Peloso Ribeiro E, Sallum EA, Sallum AW, Nociti FH Jr., Casati MZ. Semilunar coronally positioned flap or subepithelial connective tissue graft for the treatment of gingival recession: A 30-month follow-up study. *J Periodontol*. 2009;80(7):1076–1082. doi:10.1902/jop.2009.080498
8. Bittencourt S, Del Peloso Ribeiro E, Sallum EA, Nociti FH Jr., Casati MZ. Surgical microscope may enhance root coverage with subepithelial connective tissue graft: A randomized-controlled clinical trial. *J Periodontol*. 2012;83(6):721–730. doi:10.1902/jop.2011.110202
9. Tarnow DP. Semilunar coronally repositioned flap. *J Clin Periodontol*. 1986;13(3):182–185. doi:10.1111/j.1600-051x.1986.tb01456.x
10. Tarnow DP. Solving restorative esthetic dilemmas with the semilunar coronally positioned flap. *J Esthet Rest Dent*. 1994;6(2):61–64. doi:10.1111/j.1708-8240.1994.tb00834.x
11. Bittencourt S, Del Peloso Ribeiro E, Sallum EA, Sallum AW, Nociti FH Jr., Casati MZ. Root surface biomodification with EDTA for the treatment of gingival recession with a semilunar coronally repositioned flap. *J Periodontol*. 2007;78(9):1695–1701. doi:10.1902/jop.2007.060507
12. Santana RB, Mattos CM, Dibart S. A clinical comparison of two flap designs for coronal advancement of the gingival margin: Semilunar versus coronally advanced flap. *J Clin Periodontol*. 2010;37(7):651–658. doi:10.1111/j.1600-051X.2010.01582.x
13. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. *J Periodontol*. 1972;43(1):38. doi:10.1902/jop.1972.43.1.38
14. Löe H, Silness J. Periodontal disease in pregnancy I. Prevalence and severity. *Acta Odontol Scand*. 1963;21:533–551. doi:10.3109/00016356309011240
15. Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand*. 1964;22:121–135. doi:10.3109/00016356408993968
16. Paolantonio M. Treatment of gingival recessions by combined periodontal regenerative technique, guided tissue regeneration, and subpedicle connective tissue graft. A comparative clinical study. *J Periodontol*. 2002;73(1):53–62. doi:10.1902/jop.2002.73.1.53
17. Huang LH, Neiva RE, Soehren SE, Giannobile WV, Wang HL. The effect of platelet-rich plasma on the coronally advanced flap root coverage procedure: A pilot human trial. *J Periodontol*. 2005;76(10):1768–1777. doi:10.1902/jop.2005.76.10.1768
18. Yates RJ, Newcombe RG, Addy M. Dentine hypersensitivity: A randomised, double-blind placebo-controlled study of the efficacy of a fluoride-sensitive teeth mouthrinse. *J Clin Periodontol*. 2004;31(10):885–889. doi:10.1111/j.1600-051X.2004.00581.x
19. Aichelmann-Reidy ME, Yukna RA, Evans GH, Nasr HF, Mayer ET. Clinical evaluation of acellular allograft dermis for the treatment of human gingival recession. *J Periodontol*. 2001;72(8):998–1005. doi:10.1902/jop.2001.72.8.998
20. Hürzeler MB, Weng D. A single-incision technique to harvest subepithelial connective tissue grafts from the palate. *Int J Periodontics Restorative Dent*. 1999;19(3):279–287. PMID:10635174.

21. Karring T, Cumming BR, Oliver RC, Löe H. The origin of granulation tissue and its impact on postoperative results of mucogingival surgery. *J Periodontol*. 1975;46(10):577–585. doi:10.1902/jop.1975.46.10.577
22. Alkan E, Parlar A. EMD or subepithelial connective tissue graft for the treatment of single gingival recessions: A pilot study. *J Periodontal Res*. 2011;46(6):637–642. doi:10.1111/j.1600-0765.2011.01381.x
23. Keceli HG, Kamak G, Erdemir EO, Evginer MS, Dolgun A. The adjunctive effect of platelet-rich fibrin to connective tissue graft in the treatment of buccal recession defects: Results of a randomized, parallel-group controlled trial. *J Periodontol*. 2015;86(11):1221–1230. doi:10.1902/jop.2015.150015
24. Douglas de Oliveira DW, Oliveira-Ferreira F, Flecha OD, Gonçalves PF. Is surgical root coverage effective for the treatment of cervical dentin hypersensitivity? A systematic review. *J Periodontol*. 2013;84(3):295–306. doi:10.1902/jop.2012.120143
25. Cortellini P, Prato GP. Coronally advanced flap and combination therapy for root coverage. Clinical strategies based on scientific evidence and clinical experience. *Periodontol 2000*. 2012;59(1):158–184. doi:10.1111/j.1600-0757.2011.00434.x
26. Graziani F, Gennai S, Roldán S, et al. Efficacy of periodontal plastic procedures in the treatment of multiple gingival recessions. *J Clin Periodontol*. 2014;41(Suppl 15):S63–S76. doi:10.1111/jcpe.12172
27. Aydinyurt HS, Tekin Y, Ertugrul AS. The effect of enamel matrix derivatives on root coverage: A 12-month follow-up of a randomized clinical trial. *Braz Oral Res*. 2019;33:e006. doi:10.1590/1807-3107bor-2019.vol33.0006

Evaluation of the effect of injectable platelet-rich fibrin (I-PRF) in reducing the resorption of fat graft during facial lipostructure: A randomized clinical trial

Kamal Alkerdi[1,A–C], Laith Alsabek[2,C–E], Muaaz Alkhouli[3,C–E], Zuhair Al-Nerabieah[3,C–E], Hassan Jaafo[1,E,F]

[1] Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Damascus University, Syria
[2] Clinical Science Institute, National University of Ireland, Galway, Ireland
[3] Department of Pediatric Dentistry, Faculty of Dentistry, Damascus University, Syria

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):131–136

Address for correspondence
Laith Alsabek
E-mail: alsabeklaith@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on March 3, 2021
Reviewed on May 7, 2021
Accepted on May 13, 2021

Published online on March 31, 2022

Abstract

Background. Fat graft is considered to be the ideal material for soft tissue augmentation. However, its disadvantage are unpredictable outcomes due to variable resorption.

Objectives. This study is the first clinical trial to evaluate the efficacy of adding injectable platelet-rich fibrin (I-PRF) to fat graft and to compare it with the conventional fat graft in terms of absorption rate.

Material and methods. The study was designed as a double-blind, randomized, controlled clinical trial. Twenty patients were randomly assigned with regard to the right or left nasolabial folds into 2 groups ($n = 10$ in each group): group A (fat graft only); and group B (fat graft with I-PRF). Surgical lipostructure was performed in accordance with the protocols described by Coleman. The adipose tissue was extracted from the umbilical region. Then, for the I-PRF preparation, peripheral venous blood was collected into plastic tubes. The follow-up recall visits took place after 1 and 12 months. Five investigators evaluated the pre- and post-surgical intervention photographs based on the Modified Fitzpatrick Wrinkle Scale (MFWS).

Results. The nasolabial fold depth scores were recorded at each of the study phases: before the intervention (1); after 1 month (2); and after 12 months (3). There were statistically significant differences between the scores at various study phases in each group. The Mann–Whitney *U* test was used to detect differences between the 2 groups. There was no statistically significant difference between the 2 groups regarding nasolabial fold depth 1 month after the intervention ($p = 0.360$). After 12 months, however, the patients in group A showed higher nasolabial fold depth scores as compared to group B; this difference was statistically significant ($p = 0.000$).

Conclusions. The study demonstrated the efficacy of I-PRF in reducing the resorption of fat graft, following facial lipostructure.

Keywords: injectable platelet-rich fibrin, fat graft, lipostructure

Cite as
Alkerdi K, Alsabek L, Alkhouli M, Al-Nerabieah Z, Jaafo H. Evaluation of the effect of injectable platelet-rich fibrin (I-PRF) in reducing the resorption of fat graft during facial lipostructure: A randomized clinical trial. *Dent Med Probl.* 2022;59(1):131–136. doi:10.17219/dmp/136974

DOI
10.17219/dmp/136974

Introduction

Autologous fat grafting has proven to be an excellent choice of treatment for the correction of acquired and congenital facial deformities.[1] Fat graft is considered to be the ideal material for soft tissue augmentation, since fat occurs in abundance, can be easily harvested from different body parts, has host compatibility, and is non-immunogenic.[2,3] Despite these merits, fat grafting techniques have a major disadvantage, namely a high resorption rate due to their failure to stimulate neoangiogenesis. Therefore, multiple attempts are often needed to correct facial defects with fat grafting.[4]

Since post-transplant revascularization is crucial for the retention of fat graft, several methods have been suggested to stimulate neoangiogenesis. Many strategies to induce revascularization have been proposed, including the use of growth factors. Basic fibroblast growth factor (b-FGF) and vascular endothelial growth factor (VEGF) have shown promising results in promoting angiogenesis and increasing vessel density, thus enhancing graft quality and survival.[5,6] Other growth factors, such as insulin-like growth factor (IGF), erythropoietin, and platelet-derived growth factor (PDGF), have also been demonstrated to have a positive effect on the survival of fat graft.[7,8] Unfortunately, the direct use of growth factors is of limited clinical utility due to their short half-lives and the risk of tissue hyperplasia.[9]

Platelet-rich plasma (PRP) was the first plasma concentrate developed in 1998.[10] When platelets are activated, they release various bioactive proteins, which are condensed in alpha granules along with other growth factors to increase the process of tissue repair and regeneration.[11] Platelet-rich plasma requires the addition of bovine thrombin or calcium ions upon initial blood collection to activate PDGFs, which is followed by the use of anticoagulants to generate a fluid concentrate after centrifugation.[12] The drawback of using PRP is that the growth factors are released excessively after adding coagulants such that they reach their peak concentration at the first hour and decrease significantly thereafter.[13,14]

Despite the fact that PRP has shown some improvement in facial skin appearance and texture, the clinical use of PRP is limited due to the heterogeneity of the employed preparation and administration techniques, and the lack of standardization of outcome measures.[15] Moreover, there are concerns over the use of thrombin and anticoagulants, as it may interfere with wound healing by inhibiting the clotting process.[16] To overcome these challenges, a second-generation platelet concentrate, platelet-rich fibrin (PRF), was introduced.[17]

The use of PRF is a totally autologous technique that involves a one-step centrifugation process and does not require any exogenous material. Platelet-rich fibrin has demonstrated advantages when used for the soft and bony tissue healing process, and in enhancing the survival rate of fat graft.[18] In 2014, an injectable fluid form of PRF (I-PRF) was developed by reducing the force of centrifugation. The use of a lower centrifugation speed selectively augments growth factors, platelets and leukocytes within the PRF fluid matrix.[19,20] Platelets and cytokines are hitched in the I-PRF fibrin matrix after injection, resulting in the slow and gradual release of growth factors over time.[21] Injectable PRF has reportedly been used in various oral and maxillofacial procedures, such as root surface biomodification and gingival augmentation.[22,23]

To the best of our knowledge, this study is the first clinical trial to evaluate the efficacy of adding I-PRF to fat graft and to compare it with the conventional fat graft in terms of absorption rate.

Material and methods

The Ethics Committee of the Faculty of Dentistry of Damascus University, Syria, provided ethical approval for this study. Informed written consent forms that described the purpose and scope of the study were signed by the participants. The study was designed as a double-blind, randomized, controlled clinical trial. It was recorded in the German Clinical Trials Register (DRKS – Deutschen Register Klinischer Studien) (ID: DRKS00023758). The CONSORT (Consolidated Standards of Reporting Trials) statement was used; the CONSORT flow diagram illustrating the research process is presented in Fig. 1.

Sample size determination

The sample size was calculated using the G*Power software, v. 3.1 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). The significance level was set at 0.05 and the power of the study was set at 95%. The sample size was determined to be a total of 20 patients randomized for treatment group A or B (10 patients in each arm).

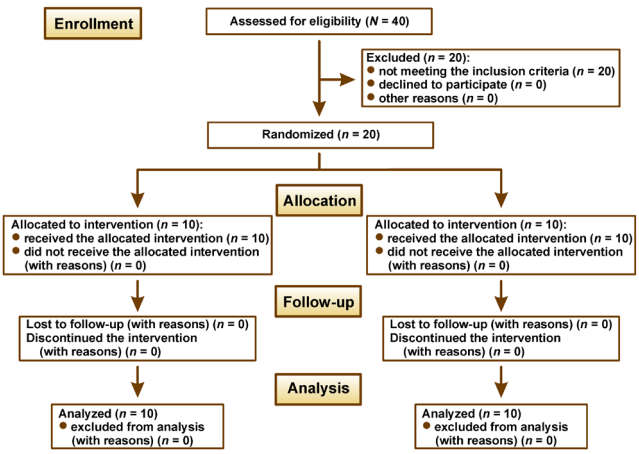

Fig. 1. Flow diagram of the study design according to the CONSORT (Consolidated Standards of Reporting Trials) 2010 Statement

Selection of participants

Twenty participants were selected from the patients attending the Department of Oral and Maxillofacial Surgery at the Faculty of Dentistry, Damascus University, according to the following inclusion and exclusion criteria as assessed by an experienced investigator:

– inclusion criteria: patients aged 18 years or older; and patients with a visible wrinkle score of 2 or 3 based on the Modified Fitzpatrick Wrinkle Scale (MFWS)[24] (moderate to deep wrinkles);
– exclusion criteria: patients under 18 years of age; patients with any systemic disease that affects the inflammatory response; patients who have previously underwent facial reconstruction surgery with the use of fat or any other filler; and patients with bleeding disorders (hemophilia, von Willebrand disease).

Allocation method

The participants were blinded to the kind of treatment applied. The units for randomization were treatment A or treatment B. An opaque envelope was used in the allocation process and each participant drew a lot from the envelope. The 2 kinds of treatment were as follows:

– treatment A (n = 10) – the injection of fat graft into the nasolabial fold; and
– treatment B (n = 10) – the injection of fat graft with I-PRF into the nasolabial fold.

Outcome measurement

The follow-up recalls were conducted after 1 and 12 months. Five investigators evaluated the pre- and post-surgical intervention photographs based on MFWS. All examiners were blinded as to which side received treatment A or B, as well as to other reviewers' responses. They were asked to provide the corresponding score from 0 to 3 based on MFWS (Table 1).

Table 1. Modified Fitzpatrick Wrinkle Scale (MFWS) (the evaluation of wrinkle depth is based on the assessors' estimation rather than a physical measurement)

Score	Discretion
0	no wrinkle; no visible wrinkle, a continuous skin line
0.5	very shallow yet visible wrinkle
1	fine wrinkle; visible wrinkle and slight indentation
1.5	visible wrinkle and clear indentation (wrinkle depth <1 mm)
2	moderate wrinkle; clearly visible wrinkle (wrinkle depth of 1–2 mm)
2.5	prominent and visible wrinkle (wrinkle depth >2 mm and <3 mm)
3	deep wrinkle; deep and furrow wrinkle (wrinkle depth >3 mm)

Fat preparation

Surgical lipostructure was performed in accordance with the protocols described by Coleman.[25] The adipose tissue was extracted from the umbilical region. The extraction was carried by means of a special aspiration nozzle with a diameter of 3 mm and a length of 15 cm, with foam ends and openings on both sides. Approximately 60 mL of fat was extracted; there were variations between the patients depending on the indication and the amount required for re-injection. The next step in the preparation process was the centrifugation of the extracted tissue. The 10-milliliter syringes were sealed and centrifugation was performed for 3 min at a speed of 3,000 rpm. Once centrifuged, the syringe contents were divided into 3 layers. The upper layer included triglycerides from the damaged adipocytes; this part is usually eliminated. The middle layer contained adipocytes, which were grafted. The bottom layer contained primarily blood debris, which was eliminated when the syringe was withdrawn.

I-PRF preparation

At the start of each treatment session, 40 mL of peripheral venous blood was collected into a sterile 10-milliliter plastic PRF tube without an anticoagulant and centrifuged immediately at room temperature, using a low relative centrifugal force of 700 rpm for 3 min.[26]

Combination of I-PRF and fat

The activation of coagulation and central forces formed 2 layers in the tube – a liquid top layer that represented I-PRF, which was drawn into a new sterile tube for use, and a lower layer that contained red blood cells, which was removed. According to Coleman's technique,[27] the resulting I-PRF was mixed with fat graft material and injected into the face. The harvested tissue was added gradually. Cannulas of various lengths and shapes were used to reduce the risk of hematoma formation, and lateral injection apertures were used to avoid injection into blood vessels.

Statistical analysis

The level of significance (p-value) and the power of the study were set at 0.05 and 90%, respectively. The Friedman test was applied to study differences in the depth of the nasolabial folds between the study phases in each group. The post hoc Wilcoxon test was used to show differences more precisely. In addition, the Mann–Whitney U test was carried out to study differences between the 2 groups. The IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA), was used to perform the statistical analysis.

Results

The sample consisted of 20 patients. Each patient was randomly assigned into one of the 2 groups: group A (fat graft only); or group B (fat graft with I-PRF). The gender and age of the participants are summarized in Table 2.

Follow-up evaluations were conducted after 1 and 12 months to record the changes in nasolabial fold depth caused by the interventions according to MFWS. The scores of the nasolabial fold depth were recorded for each of the 3 study phases. The study phases corresponded to the assessments made before the intervention (1), 1 month after the intervention (2) and 12 months after the intervention (3). The frequencies of each score for nasolabial fold depth within each group through the study phases are recorded in Table 3.

In order to study differences in nasolabial fold depth between the study phases in each group, the Friedman test was applied. It revealed that there were statistically significant differences between the study phases for each group. Table 4 presents the mean ranks for nasolabial fold depth and the Friedman test results. Pairwise comparisons with the use of the Wilcoxon test revealed that the differences between all the particular phases of the study for both treatment groups were statistically significant (Table 5). When analyzing the mean ranks for nasolabial fold depth shown in Table 4, it can be noticed that the lowest nasolabial fold depth values were recorded in study phase 2. The values were higher 1 year after the intervention, but still lower than those recorded before the intervention.

The Mann–Whitney *U* test was carried out to study differences between the 2 groups. The test showed that 1 year after the intervention, the patients in group A had higher scores for nasolabial fold depth than those in group B; this difference was statistically significant (p = 0.000). This means that the resorption rate of the fat injected was higher in group A in comparison with group B. Conversely, there was no statistically significant difference between the 2 groups regarding nasolabial fold depth 1 month after the intervention (p = 0.360) (Table 6).

Table 2. Gender and age of the study participants

Characteristics	*N* = 20
Male *n* (%)	2 (10)
Female *n* (%)	18 (90)
Mean age [years]	39.4

Table 4. Mean ranks for nasolabial fold depth and the Friedman test results

Treatment group	Mean ranks for nasolabial fold depth			χ^2 value	*p*-value
	study phase 1	study phase 2	study phase 3		
A (*n* = 10)	2.85	1.00	2.15	18.865	0.000*
B (*n* = 10)	3.00	1.05	1.95	19.538	0.000*

* statistically significant.

Table 5. Wilcoxon test results showing statistically significant differences in nasolabial fold depth between every 2 study phases for both treatment groups

Treatment group	Pairwise comparison of the study phases	*p*-value
A	phase 1 vs. phase 2	0.004*
	phase 1 vs. phase 3	0.011*
	phase 2 vs. phase 3	0.004*
B	phase 1 vs. phase 2	0.004*
	phase 1 vs. phase 3	0.005*
	phase 2 vs. phase 3	0.007*

* statistically significant.

Table 6. Differences in nasolabial fold depth between the 2 treatment groups for all study phases

Study phase	Treatment group		*p*-value
	A	B	
1	2.85	3.00	0.110
2	1.00	1.05	0.360
3	2.15	1.95	0.000*

* statistically significant.

Table 3. Frequencies of the particular scores assigned to nasolabial fold depth within each treatment group through the study phases

Study phase	Treatment group	Nasolabial depth scores							Total
		0	0.5	1	1.5	2	2.5	3	
1	A	0	0	0	0	3	4	3	10
	B	0	0	0	0	2	4	4	10
2	A	1	6	3	0	0	0	0	10
	B	5	5	0	0	0	0	0	10
3	A	0	0	0	2	4	4	0	10
	B	0	2	5	3	0	0	0	10

Study phases: 1 – before the intervention; 2 – 1 month after the intervention; 3 – 12 months after the intervention. Treatment groups: A – fat graft only; B – fat graft with injectable platelet-rich fibrin (I-PRF).

Discussion

In this study, the efficacy of I-PRF added to fat graft for nasolabial augmentation was assessed and compared with the conventional fat graft without I-PRF. After 1 month, great enhancement was noted in both groups. However, this improvement was more significant at 1 year after treatment with I-PRF.

Although there are many donor sites from which fat can be obtained, the optimal area has not yet been determined. In this study, the abdomen was selected for fat extraction. This is because it can be considered a safe and easy-to-reach site with abundant fat. Coleman's protocol of fat aspiration was implemented in this study.[27] A special aspiration nozzle with a diameter of 3 mm and a length of 15 cm, with foam ends and openings on both sides was used. According to Simonacci et al., this method has many advantages; it causes the least trauma to the donor site and preserves the viability of the adipose cells.[28]

There are many techniques that can be used for fat preparation. Coleman's protocol is the most common method, according to many studies.[29] The fat syringes were centrifuged for 3 min at a speed of 3,000 rpm. This technique is the easiest one and it aids in preserving the adipose cells.

The fan injection technique was used to inject the fatty tissue. This technique can increase the fat graft surface area and help in providing more blood supply to the graft. Cook et al. noted that injecting fat into a blood-rich area can protect the adipose tissue from subsequent necrosis.[30] A 16-gauge cannula connected to a 1-cubic centimeter syringe was used to inject the graft. This was recommended by Coleman in order to prevent the development of hematoma after injection.[3]

Platelet-rich fibrin is the effect of the progressive development of platelet-rich matrices, advantageous in terms of positive outcomes of PDGFs on tissue rejuvenation. It varies from other platelet-rich aggregates, such as PRP and plasma rich in growth factors (PRGF) in numerous aspects.[31] The PRF preparation does not necessitate any additives to prevent blood coagulation or activate the platelets. Moreover, the PRF preparation requires a very low centrifugation speed to ensure the successful apprehension of both platelets and regenerative cells, which leads to an increased concentration and a prolonged effect of growth factors. Furthermore, it has been shown that PRF has higher amounts of platelets, fibrin, growth factors, and leukocytes in comparison with PRP and PRGF, leading to a more enhanced growth factor-mediated functional outcome.[32]

The clinical use of I-PRF for facial rejuvenation has been previously reported in a few studies,[21,33,34] but the efficacy of I-PRF added to fat graft in nasolabial augmentation has not yet been studied. Hassan et al. recently reported the efficacy of I-PRF for facial rejuvenation.[35] The injectable form of PRF employed in their study was prepared using short-time and low-speed centrifugation (3 min at 700 rpm), which is similar to the preparation method used in this study. They demonstrated significant improvement in several skin characteristics, which was mirrored by significant improvement in patient satisfaction, thereby suggesting a benefit for the use of I-PRF for facial skin rejuvenation.[35]

Twelve months after the intervention, the degree of wrinkle relapse on the I-PRF side was lesser as compared to the other side in all patients. This can be attributed to the enrichment of I-PRF with growth factors, especially PDGF, which is considered to be the primary factor in wound healing and angiogenesis in the injected area.[36] Vascular endothelial growth factor is another factor that contributes to angiogenesis by encouraging the development of the basal cell layers of the endothelial tissue.[37] The effect of these growth factors on preserving blood supply to the graft could be the main reason for reducing the resorption of the graft in the treatment B group. This is in accordance with the findings of Chasan and Rahban, who reported that the most common reason for the resorption of autogenous fat graft is the impaired blood supply.[38]

Limitations

One limitation of this study is that the literature lacks research on this topic. Another limitation is that it was a single-center trial with a single ethnic group, so the results cannot be generalized until further multicenter trials with different ethnic groups are available. Finally, the limited treatment area (the nasolabial fold) could be considered yet another limitation.

Conclusions

In summary, this study demonstrated the efficacy of I-PRF in reducing the resorption of fat graft, following facial lipostructure.

Trial registration

The trial was recorded in the German Clinical Trials Register (DRKS – Deutschen Register Klinischer Studien) (ID: DRKS00023758).

Ethics approval and consent to participate

The study was approved by the institutional ethics committee at the Faculty of Dentistry of Damascus University, Syria. The participants provided informed written consent prior to the investigations.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Kamal Alkerdi https://orcid.org/0000-0001-6679-7655
Laith Alsabek https://orcid.org/0000-0003-0837-4405
Muaaz Alkhouli https://orcid.org/0000-0003-0220-3859
Zuhair Al-Nerabieah https://orcid.org/0000-0003-1843-1776
Hassan Jaafo https://orcid.org/0000-0002-0602-9678

References

1. Wetterau M, Szpalski C, Hazen A, Warren SM. Autologous fat grafting and facial reconstruction. *J Craniofac Surg*. 2012;23(1):315–318. doi:10.1097/scs.0b013e318241e1de
2. Coleman SR. Structural fat grafting: More than a permanent filler. *Plast Reconstr Surg*. 2006;118(3 Suppl):108S–120S. doi:10.1097/01.prs.0000234610.81672.e7
3. Coleman SR. Structural fat grafts: The ideal filler? *Clin Plast Surg*. 2001;28(1):111–119. doi:10.1016/s0094-1298(20)32343-9
4. Hetherington HE, Block JE. Facial fat grafting with a prototype injection control device. *Clin Cosmet Investig Dermatol*. 2013;6:201–209. doi:10.2147/ccid.s47469
5. Kao HK, Hsu HH, Chuang WY, Chang KP, Chen B, Guo L. Experimental study of fat grafting under negative pressure for wounds with exposed bone. *Br J Surg*. 2015;102(8):998–1005. doi:10.1002/bjs.9826
6. Ding SL, Zhang MY, Tang SJ, Yang H, Tan WQ. Effect of calcium alginate microsphere loaded with vascular endothelial growth factor on adipose tissue transplantation. *Ann Plast Surg*. 2015;75(6):644–651. doi:10.1097/sap.0000000000000201
7. Karschnia P, Scheuer C, Heß A, Später T, Menger MD, Laschke MW. Erythropoietin promotes network formation of transplanted adipose tissue-derived microvascular fragments. *Eur Cell Mater*. 2018;35:268–280. doi:10.22203/eCM.v035a19
8. Craft RO, Rophael J, Morrison WA, Vashi AV, Mitchell GM, Penington AJ. Effect of local, long-term delivery of platelet-derived growth factor (PDGF) on injected fat graft survival in severe combined immunodeficient (SCID) mice. *J Plast Reconstr Aesthet Surg*. 2009;62(2):235–243. doi:10.1016/j.bjps.2007.11.017
9. Xiong S, Qiu L, Zhao J, et al. The role of platelet concentrates in facial fat grafting. *Ann Plast Surg*. 2018;81(6S Suppl 1):S117–S123. doi:10.1097/SAP.0000000000001498
10. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1998;85(6):638–646. doi:10.1016/s1079-2104(98)90029-4
11. Eppley BL, Pietrzak WS, Blanton M. Platelet-rich plasma: A review of biology and applications in plastic surgery. *Plast Reconstr Surg*. 2006;118(6):147e–159e. doi:10.1097/01.prs.0000239606.92676.cf
12. Sclafani AP. Platelet-rich fibrin matrix for improvement of deep nasolabial folds. *J Cosmet Dermatol*. 2010;9(1):66–71. doi:10.1111/j.1473-2165.2010.00486.x
13. Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: Implications for wound healing. *Plast Reconstr Surg*. 2004;114(6):1502–1508. doi:10.1097/01.prs.0000138251.07040.51
14. Thor A, Franke-Stenport V, Johansson CB, Rasmusson L. Early bone formation in human bone grafts treated with platelet-rich plasma: Preliminary histomorphometric results. *Int J Oral Maxillofac Surg*. 2007;36(12):1164–1171. doi:10.1016/j.ijom.2007.05.023
15. Maisel-Campbell AL, Ismail A, Reynolds KA, et al. A systematic review of the safety and effectiveness of platelet-rich plasma (PRP) for skin aging. *Arch Dermatol Res*. 2020;312(5):301–315. doi:10.1007/s00403-019-01999-6
16. Miron RJ, Zucchelli G, Pikos MA, et al. Use of platelet-rich fibrin in regenerative dentistry: A systematic review. *Clin Oral Investig*. 2017;21(6):1913–1927. doi:10.1007/s00784-017-2133-z
17. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodont*. 2006;101(3):e45–e50. doi:10.1016/j.tripleo.2005.07.009
18. Yu P, Zhai Z, Jin X, Yang X, Qi Z. Clinical application of platelet-rich fibrin in plastic and reconstructive surgery: A systematic review. *Aesthetic Plast Surg*. 2018;42(2):511–519. doi:10.1007/s00266-018-1087-0
19. Wend S, Kubesch A, Orlowska A, et al. Reduction of the relative centrifugal force influences cell number and growth factor release within injectable PRF-based matrices. *J Mater Sci Mater Med*. 2017;28(12):188. doi:10.1007/s10856-017-5992-6
20. Choukroun J, Ghanaati S. Reduction of relative centrifugation force within injectable platelet-rich-fibrin (PRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: The first introduction to the low speed centrifugation concept. *Eur J Trauma Emerg Surg*. 2018;44(1):87–95. doi:10.1007/s00068-017-0767-9
21. Miron RJ, Fujioka-Kobayashi M, Hernandez M, et al. Injectable platelet rich fibrin (i-PRF): Opportunities in regenerative dentistry? *Clin Oral Investig*. 2017;21(8):2619–2627. doi:10.1007/s00784-017-2063-9
22. İzol BS, Üner DD. A new approach for root surface biomodification using injectable platelet-rich fibrin (I-PRF). *Med Sci Monit*. 2019;25:4744–4750. doi:10.12659/MSM.915142
23. Ozsagir ZB, Saglam E, Yilmaz BS, Choukroun J, Tunali M. Injectable platelet-rich fibrin and microneedling for gingival augmentation in thin periodontal phenotype: A randomized controlled clinical trial. *J Clin Periodontol*. 2020;47(4):489–499. doi:10.1111/jcpe.13247
24. Shoshani D, Markovitz E, Monstrey SJ, Narins DJ. The modified Fitzpatrick Wrinkle Scale: A clinical validated measurement tool for nasolabial wrinkle severity assessment. *Dermatol Surg*. 2008;34(Suppl 1):S85–S91. doi:10.1111/j.1524-4725.2008.34248.x
25. Braccini F, Dohan DM. The relevance of Choukroun's platelet rich fibrin (PRF) during facial aesthetic lipostructure (Coleman's technique): Preliminary results [in French]. *Rev Laryngol Otol Rhinol (Bord)*. 2007;128(4):255–260. PMID:18320933.
26. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized platelet-rich fibrin with the low-speed concept: Growth factor release, biocompatibility, and cellular response. *J Periodontol*. 2017;88(1):112–121. doi:10.1902/jop.2016.160443
27. Pu LL, Coleman SR, Cui X, Ferguson RE, Vasconez HC. Cryopreservation of autologous fat grafts harvested with the Coleman technique. *Ann Plast Surg*. 2010;64(3):333–337. doi:10.1097/SAP.0b013e3181b022cb
28. Simonacci F, Bertozzi N, Grieco MP, Grignaffini E, Raposio E. Procedure, applications, and outcomes of autologous fat grafting. *Ann Med Surg (Lond)*. 2017;20:49–60. doi:10.1016/j.amsu.2017.06.059
29. Strong AL, Cederna PS, Rubin JP, Coleman SR, Levi B. The current state of fat grafting. *Plast Reconstr Surg*. 2015;136(4):897–912. doi:10.1097/PRS.0000000000001590
30. Cook T, Nakra T, Shorr N, Douglas RS. Facial recontouring with autogenous fat. *Facial Plast Surg*. 2004;20(2):145–147. doi:10.1055/s-2004-861755
31. Wang X, Zhang Y, Choukroun J, Ghanaati S, Miron RJ. Effects of an injectable platelet-rich fibrin on osteoblast behavior and bone tissue formation in comparison to platelet-rich plasma. *Platelets*. 2018;29(1):48–55. doi:10.1080/09537104.2017.1293807
32. Fan Y, Perez K, Dym H. Clinical uses of platelet-rich fibrin in oral and maxillofacial surgery. *Dent Clin North Am*. 2020;64(2):291–303. doi:10.1016/j.cden.2019.12.012
33. Varela HA, Souza JC, Nascimento RM, et al. Injectable platelet rich fibrin: Cell content, morphological, and protein characterization. *Clin Oral Investig*. 2019;23(3):1309–1318. doi:10.1007/s00784-018-2555-2
34. Fujioka-Kobayashi M, Schaller B, De Almeida Barros Mourão CF, Zhang Y, Sculean A, Miron RJ. Biological characterization of an injectable platelet-rich fibrin mixture consisting of autologous albumin gel and liquid platelet-rich fibrin (Alb-PRF). *Platelets*. 2021;32(1):74–81. doi:10.1080/09537104.2020.1717455
35. Hassan H, Quinlan DJ, Ghanem A. Injectable platelet-rich fibrin for facial rejuvenation: A prospective, single-center study. *J Cosmet Dermatol*. 2020;19(12):3213–3221. doi:10.1111/jocd.13692
36. Kim J, Ha Y, Kang NH. Effects of growth factors from platelet-rich fibrin on the bone regeneration. *J Craniofac Surg*. 2017;28(4):860–865. doi:10.1097/SCS.0000000000003396
37. Cabaro S, D'Esposito V, Gasparro R, et al. White cell and platelet content affects the release of bioactive factors in different blood-derived scaffolds. *Platelets*. 2018;29(5):463–467. doi:10.1080/09537104.2017.1319046
38. Chasan PE, Rahban SR. Presacral donor site for lip augmentation. *Aesthetic Plast Surg*. 2000;24(1):31–33. doi:10.1007/s002669910006

Revolution in modern teaching in dentistry since the appearance of the COVID-19 pandemic: A review

Sergio Marcelo Delgado-Castillo[1,A–F], Sayuri Miguel-Soto[1,A–F], Katherine Joselyn Atoche-Socola[1,A–F], Luis Ernesto Arriola-Guillén[2,3,A–F]

[1] School of Dentistry, Scientific University of the South (Universidad Científica del Sur – UCSUR), Lima, Peru
[2] Department of Orthodontics, Scientific University of the South (Universidad Científica del Sur – UCSUR), Lima, Peru
[3] Department of Oral and Maxillofacial Radiology, Scientific University of the South (Universidad Científica del Sur – UCSUR), Lima, Peru

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):137–141

Address for correspondence
Luis Ernesto Arriola-Guillén
E-mail: luchoarriola@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on April 6, 2021
Reviewed on August 17, 2021
Accepted on August 23, 2021

Published online on March 31, 2022

Abstract

Dentistry schools have attempted to overcome the challenges imposed by the coronavirus disease 2019 (COVID-19) pandemic through teaching via the Internet with the use of virtual platforms, designed to simulate face-to-face interaction and counteract the social isolation affecting the integral development of students. This review searched the main health databases, including MEDLINE via PubMed, Scopus and LILACS, and selected 31 articles to proceed with the research. During the pandemic, platforms such as Facebook, Instagram, YouTube, Zoom, Google Meet, and other similar tools allowed teachers to develop dynamic slides and dental models to simulate procedures, which played an important role in the course of mainly theoretical classes. In addition, applications such as WhatsApp allowed the rapid acquisition and sharing of useful information on a specific topic. While virtual resources facilitate the learning process through generating interest as well as providing accurate, necessary, valuable, and easily accessible information, which is constantly updated, the disadvantages of remote learning include the lack of instruments, infrastructure and materials, apart from supervision, to promote personal development and progressive evolution to directly treat patients. Another issue with regard to virtual learning is whether within a short period of time students can achieve a comparable level of practical skills as in the case of the conventional learning. In conclusion, the current pandemic has changed not only the use of technology in education, but also educational strategies for the future.

Keywords: virtual learning, COVID-19, dentistry

Cite as
Delgado-Castillo SM, Miguel-Soto S, Atoche-Socola KJ, Arriola-Guillén LE. Revolution in modern teaching in dentistry since the appearance of the COVID-19 pandemic: A review. *Dent Med Probl.* 2022;59(1):137–141. doi:10.17219/dmp/141522

DOI
10.17219/dmp/141522

Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by a viral agent identified as a novel betacoronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] The pandemic caused by COVID-19 has had an unprecedented effect on global health and has led to important alterations in many activities, including education.[2] Schools of dentistry have attempted to overcome the current situation through teaching via the Internet with the use of virtual platforms, designed to simulate face-to-face interaction and counteract the social isolation affecting the integral development of students.[3,4]

The global strategy aimed at reducing the spread of COVID-19 included the promotion of social distancing as well as the suspension of instruction in the classroom in order to avoid the crowding of too many people in enclosed spaces. Up to November 24, 2020, more than 61.2 million cases and 1.44 million deaths had been reported worldwide. Moreover, more than 900 million students at all levels of education, including higher education, had been affected.[5–7]

The face-to-face educational activities of undergraduate and postgraduate schools of dentistry have been interrupted, leaving teachers and students to adapt to social distancing. The teaching of dentistry involves face-to-face settings with specialists and students to enable the evaluation of the signs and symptoms of oral diseases, to achieve a correct diagnosis, and to develop an adequate and individualized treatment plan.[8,9]

In these times, quarantines and social isolation could happen again. Thus, some virtual solutions are being implemented to continue the teaching activities. The electronic tools used in dental education centers include Moodle, Zoom, Jitsi, and Webex, which are interesting alternatives to classrooms. There are also other tools, such as Microsoft Teams, Google Meet, Google Classroom, and Google Hangout, which facilitate live activities, involving hundreds of participants simultaneously. The presenter's screen can be shared, allowing various didactic actions. In addition, classes can be recorded and stored on Google Drive, providing students with the possibility to learn later on.[10]

All of these applications help provide the theoretical content, but do not enable participation in laboratory, preclinical and clinical activities.[11–13] Teaching with the use of the currently most popular social networks, such as Facebook, Instagram, WhatsApp, and YouTube, offers more options and is more dynamic as compared to other student platforms.[14–18] Rapid and ongoing communication between cities and countries by means of social networks provides the current context, facilitating a search for answers to the unsolved questions based on alternative virtual resources. While virtual platforms may be considered a lifesaver during the time of a pandemic by connecting teachers with students, there are also some disadvantages that should be taken into account. Students may find it difficult to concentrate on virtual education. Besides, some platforms lack filters that control students' attendance to theoretical sessions; therefore, students may only partially attend classes.[19–21]

This study aimed to determine, analyze and compare modern virtual learning/teaching based on technology vs. traditional learning/teaching. The secondary objectives were to analyze the different modern teaching tools in dentistry established during the COVID-19 pandemic, to describe resources that are most in demand at universities, to compare the information available on research platforms and social networks, and to assess the speed of the dynamic, creative sharing of knowledge that attracts the full attention of students.

Methodology

This research was based on the review of the scientific articles found on the Internet in the main heath databases. The search was carried out from July to November 2020, without limitations as to the year of publication. The articles were similar in terms of content and in the approach to the problem. Virtual tools were used, starting with the Google Scholar search engine, followed by a search for articles in the United States National Library of Medicine (MEDLINE via PubMed), Scopus and LILACS as the main sources of information. The following search terms were used: 'dentistry' ('orthodontics', 'endodontics', 'periodontics', 'pediatric dentistry', 'oral rehabilitation', and 'maxillofacial surgery'), 'virtual education', 'virtual learning', 'COVID-19', and 'SARS-CoV-2'. Observational studies, analytical and descriptive research as well as clinical trials were included. Case reports, editorials, opinion articles, literature review articles, and systematic reviews were excluded. For the inclusion of studies, the analysis was carried out in 2 phases. In the 1st phase, observational studies were analyzed. In the 2nd phase, analytical and descriptive research, and clinical trials were evaluated. The selected articles were reviewed by 2 investigators (KJAS and LEAG). Finally, following rigorous systematic selection, 31 articles were identified and included in this review (Fig. 1). All studies had a moderate risk of bias according to the Risk Of Bias In Non-randomized Studies – of Interventions (ROBINS-I) tool.

Future vision of virtual platforms in dentistry

By 2022, face-to-face classes are planned for both theoretical and practical classes. However, guided practice

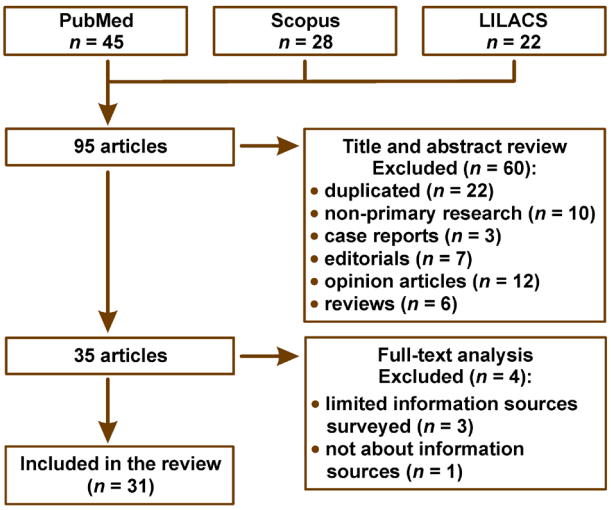

Fig. 1. Flow chart for the selection of studies

is needed to develop the operator's skills also by virtual means in order to avoid imbalance between the simulated and clinical interventions. To prepare students to adequately manage and perform treatment without complications, teaching with the use of objective videos, slides for the recognition of instruments and models that resemble work with real patients have been proposed.

Students must take advantage of the digital era and the Internet by using the immediately available virtual resources to facilitate and promote dentistry learning based on scientific evidence. Scientific websites as well as multimedia resources, like videos on YouTube, Facebook and Instagram, can clarify a concept, provide in-depth information on a specific topic or reinforce an idea. These resources are able to capture the attention of young people while providing new knowledge in a different way than the conventional university teaching. Therefore, students' schedules should include the use of virtual tools, such as online platforms and social networks, to thereby motivate students to do research, and consequently expand their knowledge beyond the lessons learned.[21–23]

Since online learning should be combined with the conventional learning, virtual tools must not replace the use of physical resources, including books, encyclopedias and atlases. From the student's perspective, a suitable environment must be created to avoid distractions and connection problems, and to optimize results. The space equipped with technological tools must be considered and the time granted by the university to complete various academic tasks must be maximized.

For all of the above, in the near future, universities should complement face-to-face classes with virtual hours to improve the level of education and to ensure the coexistence of both learning/teaching methods. One method should not replace the other, but rather a new approach should enrich the study routine, awakening new interests by handling large amounts of information.

In this era of globalization, today's students have a great advantage over those of previous decades. The Internet facilitates learning, as it generates interest as well as provides accurate, necessary, valuable, and readily available information, which is constantly updated. Due to the accessibility of technology and the speed of its diffusion, students can learn, understand, and put into practice new techniques, and gain knowledge for their long-term development as future professionals.

Use of virtual tools in dental education

In the schedules prepared by universities to carry out virtual classes, various platforms, repositories and scientific websites are needed to develop the competencies imposed in the curriculum with respect to the requirements of the national quarantine. Students can make the most of the Internet, exploring its resources. Different platforms can be analyzed for different purposes, with the main objective being virtual learning. For example, the WhatsApp application allows the sharing of information on a specific topic, whether it be data, new knowledge or news, between two or more people.[24]

During the pandemic, platforms such as Zoom, Google Meet, and other similar tools allowed interaction among the collective of students, thus playing an important role in the development of mainly theoretical classes, generating meetings, sharing ideas and knowledge, and effectively explaining the concepts of the topic to be further investigated by students. However, the question is: How can such platforms be efficiently used to conduct practical classes? The first obstacle in running practical classes from home is the lack of instruments, infrastructure and materials, apart from supervision, to promote personal development and progressive evolution to directly treat patients. Another issue with regard to virtual learning is whether within a short period of time students are able to achieve a comparable level of practical skills as in the case of the conventional learning.[24,25]

Theoretical knowledge must be balanced with practical skills, regardless of the kind of learning employed – face-to-face or through virtual platforms, social networks and applications. Face-to-face learning should be prioritized, in compliance with safety protocols, and all courses and laboratory schedules should be reinforced to complement the knowledge acquired in the virtual classroom. Virtual platforms have been a valuable tool in continuing university studies, and despite the return of face-to-face classes, the use of mobile devices and online platforms should be encouraged with the aim of expanding students' knowledge.[25]

Advantages of using virtual platforms

Learning evolves with the provision of alternative choices, with its purposes, procedures, means, and structure being defined by both students and teachers. Currently, students and teachers are developing immediate communication. In this way, virtual education is implemented, in which students learn online through various platforms, such as Zoom, Google Meet, Google Drive, and Google Classroom, among others. Taking this into account, it is necessary to discuss the advantages of virtual teaching and learning.[26]

Through the use of different platforms, virtual education saves time for students as well as teachers by not having to travel to the work or study location. Virtual education also allows the learning schedules to be flexible. In addition, the use of mobile devices has been again highlighted in the virtual setting, further breaking down the space and time barriers.[27]

These platforms allow the application of different individual and collaborative e-learning strategies, and have a huge capacity. Zoom in its free version allows up to 100 students to participate in the lectures online and 1,000 in the paid version. It facilitates the sharing of knowledge and helps build relationships, thus promoting social communication in the environment of the automatically and collaboratively programmed activities. Moreover, with the use of a laptop and a reliable Internet connection, learning can be comfortably accomplished anywhere.[25]

Another advantage of the use of virtual platforms is the speed with which students can connect, communicate, submit work, and download or view material, although this is related to the speed of the Internet, and the device and platform used. Videoconferencing with the Zoom or Google Meet platforms has been assessed positively for allowing synchronous responses to teachers' queries without delays.[26]

Google Drive and Google Classroom are some of the tools most commonly used by students and teachers due to the ease of uploading and downloading files and information to/from the platform. In addition, through the Classroom platform, teachers can quickly send university students extra materials, such as reading excerpts, presentations, texts, and reviews, before starting classes. These virtual platforms aim to improve communication and workflow between teachers and students.[27]

Disadvantages of virtual classes due to the pandemic

The use of virtual platforms requires that students have permanent access to computer media, and at least a desktop or laptop connected to the Internet. Students and teachers need to know how to use office tools, and since students have to sit in front of a computer for hours, they also must make a greater effort to avoid being distracted or carrying out other activities that could compete with their classes.[28]

Other important disadvantages of online learning include technical problems, such as the unexpected closure of the platforms in the middle of the class, while filling in a questionnaire or during exams. In such situations, students often do not know how to act, since disturbances may be related to the platforms, the Internet, or problems with the compatibility of browsers or the operating systems. These drawbacks can cause frustration on the part of students and result in the interruption of learning.[29]

The location chosen to participate in virtual classes must be adequate and orderly to enhance concentration, since bedrooms may distract students or even induce sleepiness.[30]

Virtual classes reduce the social relationships established in the classroom. Students cannot socialize with their peers beyond the mere education. The lack of interpersonal relations leads to frustration. Studying online is a very lonely activity, and students may need direct contact with the teacher to solve doubts or practice using physical tools, e.g., in laboratories, as the practical skills required for performing dentistry have been greatly affected by the pandemic.[31]

Limitations

Although the results of this study produce valuable information for dentistry, they should be evaluated with caution, because the quality assessment revealed a moderate risk of bias of the included studies. Likewise, their external validity did not allow the results to be generalized. More studies comparing traditional methods with the methods adapted due to the pandemic should be carried out.

Conclusions

Throughout the COVID-19 pandemic, modern teaching in dentistry has continued to evolve efficiently with the use of virtual tools, and although clinical practice requires contact face-to-face, virtual learning will still be employed when everyday life returns to normal. With the aim of continuing educational activities, students have been taught dentistry techniques via virtual platforms, and this has been widely accepted by both students and teachers for their ease of handling, among other advantages. Thus, despite the challenges presented by the pandemic, dentistry students have adapted to new reality, combining learning in the classroom and at a distance. The current pandemic has not only changed the use of technology in education, but also educational strategies for the future.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Sergio Marcelo Delgado-Castillo https://orcid.org/0000-0002-8115-644X
Sayuri Miguel-Soto https://orcid.org/0000-0002-2093-5802
Katherine Joselyn Atoche-Socola https://orcid.org/0000-0001-7422-1621
Luis Ernesto Arriola-Guillén https://orcid.org/0000-0003-0010-5948

References

1. Aulakh G, Wanis C, Wilson G, Moore R. The impact of COVID-19 on oral surgery training. *Oral Surg*. 2021. doi:10.1111/ors.12612
2. Kavadella A, Kossioni AE, Tsiklakis K, et al. Recommendations for the development of e-modules for the continuing professional development of European dentists. *Eur J Dent Educ*. 2013;17(Suppl 1):45–54. doi:10.1111/eje.12039
3. Munro V, Morello A, Oster C, et al. E-learning for self-management support: Introducing blended learning for graduate students – a cohort study. *BMC Med Educ*. 2018;18(1):219. doi:10.1186/s12909-018-1328-6
4. Aguilar-Gálvez D, Noal FC, de Araujo FB, Arriola-Guillén LE. Virtual learning object: An asynchronous solution for virtual learning in dentistry post COVID-19. *J Dent Educ*. 2020. doi:10.1002/jdd.12439
5. Iyer P, Aziz K, Ojcius DM. Impact of COVID-19 on dental education in the United States. *J Dent Educ*. 2020;84(6):718–722. doi:10.1002/jdd.12163
6. Tubelo RA, Castelo Branco VL, Dahmer A, Werner Samuel SM, Collares FM. The influence of a learning object with virtual simulation for dentistry: A randomized controlled trial. *Int J Med Inform*. 2016;85(1):68–75. doi:10.1016/j.ijmedinf.2015.11.005
7. Machado RA, Ferreti Bonan PR, da Cruz Perez DE, Martelli H Jr. COVID-19 pandemic and the impact on dental education: Discussing current and future perspectives. *Braz Oral Res*. 2020;34:e083. doi:10.1590/1807-3107bor-2020.vol34.0083
8. Elledge R, Williams R, Fowell C, Green J. Maxillofacial education in the time of COVID-19: The West Midlands experience. *Br J Oral Maxillofac Surg*. 2022;60(1):52–57. doi:10.1016/j.bjoms.2020.07.030
9. de Barros Silva PG, Lima de Oliveira CA, Fontes Borges MM, et al. Distance learning during social seclusion by COVID-19: Improving the quality of life of undergraduate dentistry students. *Eur J Dent Educ*. 2021;25(1):124–134. doi:10.1111/eje.12583
10. Holloway JA, Johnsen DC, Syrbu J. Student performance comparisons for a critical thinking skill set (technology decision-making) for classroom and remote (Zoom) facilitation. *J Dent Educ*. 2021;85(3):379–382. doi:10.1002/jdd.12443
11. Pather N, Blyth P, Chapman JA, et al. Forced disruption of anatomy education in Australia and New Zealand: An acute response to the COVID-19 pandemic. *Anat Sci Educ*. 2020;13(3):284–300. doi:10.1002/ase.1968
12. Huynh R. The role of e-learning in medical education. *Acad Med*. 2017;92(4):430. doi:10.1097/ACM.0000000000001596
13. Alshiekhly U, Arrar R, Barngkgei I, Dashash M. Facebook as a learning environment for teaching medical emergencies in dental practice. *Educ Health (Abingdon)*. 2015;28(3):176–180. doi:10.4103/1357-6283.178609
14. Iwanaga J, Loukas M, Dumont AS, Tubbs RS. A review of anatomy education during and after the COVID-19 pandemic: Revisiting traditional and modern methods to achieve future innovation. *Clin Anat*. 2021;34(1):108–114. doi:10.1002/ca.23655
15. Rekawek P, Rice P, Panchal N. The impact of COVID-19: Considerations for future dental conferences. *J Dent Educ*. 2020;84(11):1188–1191. doi:10.1002/jdd.12330
16. Terhune KP, Choi JN, Green JM, et al. Ad astra per aspera (Through hardships to the stars): Lessons learned from the first national virtual APDS meeting, 2020. *J Surg Educ*. 2020;77(6):1465–1472. doi:10.1016/j.jsurg.2020.06.015
17. Srivastava V, Pandey V, Tiwari P, Patel S, Ansari MA, Shukla VK. Utility of real-time online teaching during COVID era among surgery postgraduates. *Indian J Surg*. 2020:1–7. doi:10.1007/s12262-020-02592-2
18. Siliquini R, Ceruti M, Lovato E, et al. Surfing the Internet for health information: An Italian survey on use and population choices. *BMC Med Inform Decis Mak*. 2011;11:21. doi:10.1186/1472-6947-11-21
19. Rajeh MT, Sembawa SN, Nassar AA, Al Hebshi SA, Aboalshamat KT, Badri MK. Social media as a learning tool: Dental students' perspectives. *J Dent Educ*. 2021;85(4):513–520. doi:10.1002/jdd.12478
20. Cayo-Rojas CF, Agramonte-Rosell RDLC. Challenges of virtual education in dentistry in times of COVID-19 pandemic [in Spanish]. *Rev Cubana Estomatol*. 2020;57(3):e3341. http://repositoriobibliotecas.uv.cl/handle/uvscl/2013. Accessed April 1, 2021.
21. Durán Ojeda G. Preclinical simulation courses in dental education with COVID-19 pandemic [in Spanish]. *ODOVTOS-Int J Dental Sc*. 2020;22(2):11–13. doi:10.15517/IJDS.2020.41496
22. Barros de Oliveira ML, Verner FS, Kamburoğlu K, Nogueira Silva JN, Junqueira RB. Effectiveness of using a mobile app to improve dental students' ability to identify endodontic complications from periapical radiographs. *J Dent Educ*. 2019;83(9):1092–1099. doi:10.21815/JDE.019.099
23. Soltanimehr E, Bahrampour E, Imani MM, Rahimi F, Almasi B, Moattari M. Effect of virtual versus traditional education on theoretical knowledge and reporting skills of dental students in radiographic interpretation of bony lesions of the jaw. *BMC Med Educ*. 2019;19(1):233. doi:10.1186/s12909-019-1649-0
24. Huang TK, Yang CH, Hsieh YH, Wang JC, Hung CC. Augmented reality (AR) and virtual reality (VR) applied in dentistry. *Kaohsiung J Med Sci*. 2018;34(4):243–248. doi:10.1016/j.kjms.2018.01.009
25. Ayoub A, Pulijala Y. The application of virtual reality and augmented reality in oral & maxillofacial surgery. *BMC Oral Health*. 2019;19(1):238. doi:10.1186/s12903-019-0937-8
26. Ferro AS, Nicholson K, Koka S. Innovative trends in implant dentistry training and education: A narrative review. *J Clin Med*. 2019;8(10):1618. doi:10.3390/jcm8101618
27. El Tantawi MM, Abdelsalam MM, Mourady AM, Elrifae IM. e-assessment in a limited-resources dental school using an open-source learning management system. *J Dent Educ*. 2015;79(5):571–583. PMID:25941151.
28. Albuha Al-Mussawi RM, Farid F. Computer-based technologies in dentistry: Types and applications. *J Dent (Tehran)*. 2016;13(3):215–222. PMID:28392819. PMCID:PMC5376549.
29. Ghai S. Are dental schools adequately preparing dental students to face outbreaks of infectious diseases such as COVID-19? *J Dent Educ*. 2020;84(6):631–633. doi:10.1002/jdd.12174
30. Halpin PA, Lockwood MK. The use of Twitter and Zoom videoconferencing in healthcare professions seminar course benefits students at a commuter college. *Adv Physiol Educ*. 2019;43(2):246–249. doi:10.1152/advan.00017.2019
31. Wootton AR, McCuistian C, Legnitto Packard DA, Gruber VA, Saberi P. Overcoming technological challenges: Lessons learned from a telehealth counseling study. *Telemed J E Health*. 2020;26(10):1278–1283. doi:10.1089/tmj.2019.0191

Effect of conscious sedation use on anxiety reduction, and patient and surgeon satisfaction in dental implant surgeries: A systematic review and meta-analysis

Reza Pourabbas[1,A,E], Nazgol Ghahramani[2,B,C], Mehrnoosh Sadighi[1,D–F], Fatemeh Pournaghi Azar[3,B,C], Morteza Ghojazadeh[3,B,C]

[1] Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Iran
[2] Postgraduate student of pediatric dentistry, Faculty of Dentistry, Ahvaz Jundishapur University of Medical Sciences, Iran
[3] Research Center for Evidence-Based Medicine, Faculty of Medicine, Tabriz University of Medical Sciences, Iran

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl.* 2022;59(1):143–149

Address for correspondence
Mehrnoosh Sadighi
E-mail: mehrnoosh1378@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on March 14, 2021
Reviewed on July 28, 2021
Accepted on September 1, 2021

Published online on March 31, 2022

Abstract

Demand for dental implants has increased in recent years and the use of conscious sedation for this type of surgery can be of great benefit. Therefore, the aim of this systematic review was to evaluate the scientific literature related to the effect of conscious sedation on the reduction of anxiety, and patient and surgeon satisfaction.

The Embase, PubMed, ProQuest, Scopus, Ovid, and Cochrane databases were searched without limitations. According to the inclusion and exclusion criteria determined for the study, 10 articles were selected for the final review after several screening stages. These studies were reviewed in their full-text form by the research team and the intended data was extracted. The risk of bias was assessed for each of the selected articles.

Five studies were ultimately included. Two of the them compared local anesthesia and conscious sedation, while the others compared the consequences of different types of conscious sedation. The anxiety reduction and patient and surgeon satisfaction data was collated. Midazolam was the most frequently used agent.

After a thorough review of the final articles extracted based on the study protocol, it was concluded that the use of conscious sedation during implant surgery reduces patient anxiety, and also increases the satisfaction of the patient and the surgeon.

Keywords: dental implant, conscious sedation, hemodynamic, satisfaction, anxiety, systematic review

Cite as
Pourabbas R, Ghahramani N, Sadighi M, Pournaghi Azar F, Ghojazadeh M. Effect of conscious sedation use on anxiety reduction, and patient and surgeon satisfaction in dental implant surgeries: A systematic review and meta-analysis. *Dent Med Probl.* 2022;59(1):143–149. doi:10.17219/dmp/141868

DOI
10.17219/dmp/141868

Introduction

Oral surgery, including implant surgery, is usually performed with local anesthesia, either local infiltration or block anesthesia. While local anesthesia can provide adequate analgesia, the time required for extensive and complicated treatment (up to 3 hours' duration) may make it unpleasant for the patient, who is completely aware of the procedure and must remain relatively still with their mouth open for most of the time.[1] When a complicated and long implant surgery is anticipated, intravenous conscious sedation (IVCS) has become a logical and viable option.[2] Conscious sedation is a drug-induced condition in which the conscious patient is rendered free of fear, anxiety and apprehension while remaining comfortably relaxed.[3] When administering intravenous sedation, the operator aims to achieve a predetermined goal, namely an adequate level of sedation, which enables dental treatment to be carried out safely and with the minimum amount of distress for the patient.[1] Conscious sedation is not a method of pain control,[3] but it can result in better patient anxiety control and an increase in cooperation, facilitating the intervention for the patient and the surgeon.[4]

Anxiety is defined as a state of apprehension and physical tension combined with the activation of the autonomic nervous system. It is a common emotional reaction to fear experienced by patients before dental treatment or the application of a local anesthetic.[1] Anxiety control before and during dental procedures is important to ensure safety, and to promote overall patient and surgeon satisfaction.[4,5] Unfortunately, the amount of research investigating the effect of IVCS on anxiety in patients undergoing dental implant surgery, as well as on patient and surgeon satisfaction, is still limited.[2,6,7]

Therefore, the aim of this systematic review was to review the literature regarding the effect of conscious sedation on the reduction of anxiety, and patient and surgeon satisfaction in dental implant surgery.

Methods

Study design

A comprehensive literature review of research databases, including Embase, PubMed, ProQuest, Scopus, and Ovid, was conducted. The first author (RP), assisted by a research librarian, generated a literature search strategy and searched the databases. The literature searches were carried out from February 2018 to May 2019. On May 27, 2019, a final search update was made in all databases. The search strategy included a combination of MeSH (Medical Subject Headings) and free keywords.

Inclusion and exclusion criteria

The study selection process used the Population, Intervention (or Exposure), Comparison, and Outcome (PICO) framework to clarify the search.[8]

Population: Patients with implant surgeries (healthy patients aged ≥18 years) participating in randomized controlled trials (RCTs).

Intervention: IVCS.

Comparison: Local anesthesia.

Outcome: Anxiety, patient and surgeon satisfaction.

The search, selection and assessment processes were performed in 4 steps according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flow diagram shown in Fig. 1. The steps were as follows: systematic literature search; removal of duplicates; identification of potentially relevant articles based on the title and abstract; and full-text screening. The first and second authors (RP and NG), assisted by a librarian, prepared the search string for the electronic search. For more precise results, a manual search was performed among the references of the gathered articles. Also, the research team contacted study authors to obtain additional information, if necessary. The first author (RP) screened the titles and abstracts, and the first and second authors (RP and NG) separately read the full text of the remaining articles for inclusion or exclusion in the review. The selected articles were appraised based on Cochrane's tool for assessing the risk of bias in randomized trials.[9] The articles were appraised by two members of the research team, and a third arbitrator was consulted when there were points of disagreement. The included studies were reviewed and the intended data was extracted by two

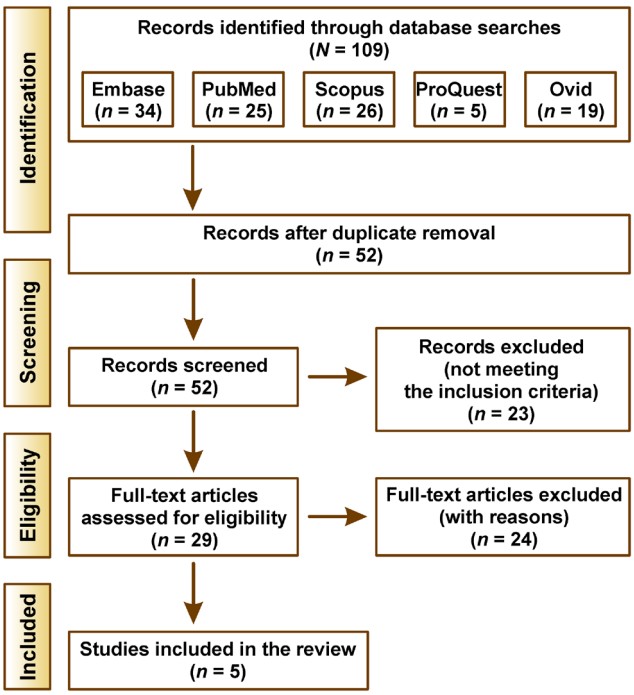

Fig. 1. PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flow diagram of the study

reviewers independently, using a standardized data extraction tool. The results were reported as percentages and as mean ± standard deviation (*M* ±*SD*).

Results

The PRISMA[10] flow chart summarizes the selection of articles included in the systematic review (Fig. 1); 109 articles were found through the searches in the databases. Once duplicates were removed, 52 articles remained. After screening the titles and abstracts, 23 articles were excluded based on the inclusion criteria from PICO and 29 articles remained. After full-text screening, 5 studies were left for inclusion in the systematic review. The reasons for the exclusion of the 24 articles during full-text screening were as follows: 5 of the articles were not written in English; the full text of 3 articles was not accessible electronically; 4 articles assessed oral or inhalation sedation instead of intravenous sedation; 5 of the articles included patients assigned to class 2 or upper according to the American Society of Anesthesiologists (ASA) physical status classification[11]; and the outcome of 7 studies did not fulfill the criteria listed in PICO.

Anxiety

Four studies[2,4,6,12] evaluated anxiety in patients undergoing IVCS. The descriptive data of the included studies for anxiety reduction is shown in Table 1. Midazolam and fentanyl were used for sedation in 2 cases,[4,6] while midazolam only was used in 1 study.[2] Kaviani and Ghoreishain compared 2 groups (midazolam and fentanyl vs. midazolam and ketamine).[12] Three of the studies reported anxiety as high, moderate or low.[4,6,12] One study reported only high anxiety.[2] Table 2 and Fig. 2 depict the pooled adjusted estimates of the association between IVCS and the anxiety levels.

High anxiety

Four studies that evaluated high anxiety in patients with IVCS were assigned to meta-analysis; a total of 473 patients from the eligible studies were analyzed. The heterogeneity between the studies was statistically significant

Table 1. Descriptive data of the included studies regarding anxiety reduction

Study	Sample size	Sedation protocol	Anxiety %			Preoperative anxiety score *M* ±*SD*
			low	moderate	high	
McCrea[2] 2015	173	midazolam	–	–	44	M (*n* = 64): 9.19 ±4.21 F (*n* = 109): 11.86 ±5.76
Gonzáles-Lemmonier et al.[4] 2010	90	midazolam (0.05 mg/kg) + fentanyl (1 µg/kg)	27.8	50.0	22.2	high in F
Bovaira et al.[6] 2017	180	midazolam (0.05 mg/kg) + fentanyl (1 mg/kg)	27.8	56.1	16.1	M (*n* = 104): 9.73 ±3.37 F (*n* = 76): 8.51 ±3.65
Kaviani and Ghoreishain[12] 2014	group A: 15 group B: 15	group A: midazolam (1 mg) + fentanyl (50 µg) group B: midazolam (1 mg) + ketamine (50 mg)	group A: 20.0 group B: 40.0	group A: 60.0 group B: 46.7	group A: 20.0 group B: 13.3	–

M – mean; *SD* – standard deviation; M – male; F – female.

Table 2. Pooled adjusted estimates of the association between intravenous conscious sedation (IVCS) and the anxiety levels

Anxiety	Model	Number of studies	Effect size and 95% *CI*			Null-hypothesis two-tailed test		Heterogeneity			
			point estimate	lower limit	upper limit	*Z*-value	*p*-value	*Q*-value	df (*Q*)	*p*-value	I^2
High	random-effects	4	0.250	0.128	0.430	–2.632	0.008*	34.61	3	<0.001*	91.33
Moderate	fixed-effects	3	0.544	0.485	0.601	1.469	0.142	1.10	2	0.577	0.00
Low	fixed-effects	3	0.274	0.226	0.329	–7.318	0.000*	0.43	2	0.807	0.00

CI – confidence interval; df – degrees of freedom; * statistically significant.

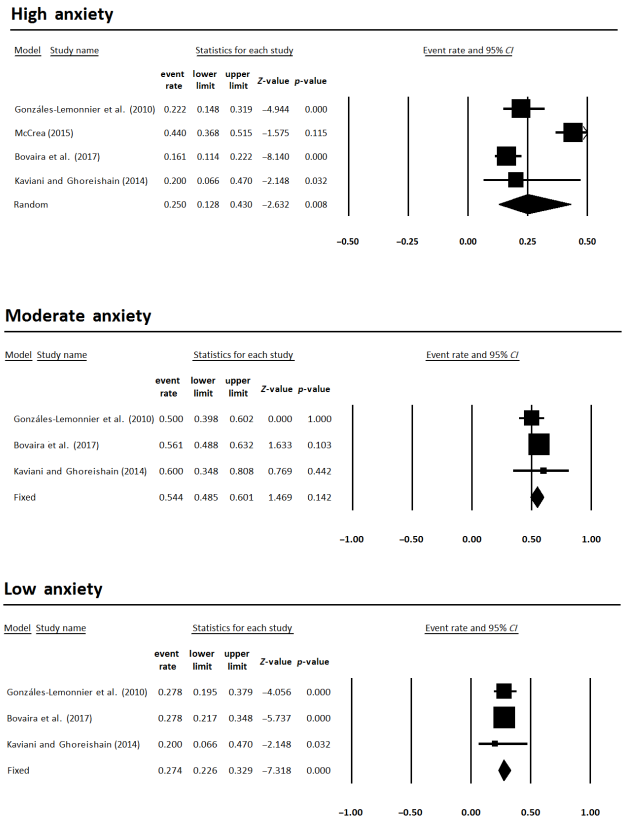

Fig. 2. Pooled adjusted estimates of the association between intravenous conscious sedation (IVCS) and the anxiety levels

(Q = 34.61; p < 0.001; I^2 = 91.33). The meta-analysis results with the random-effects model indicated a high anxiety score of 25.0%, which was statistically significant (pooled event rate: 0.250; 95% *CI* (confidence interval): 0.128–0.430; p = 0.008).

Moderate anxiety

Three studies that evaluated moderate anxiety in patients with IVCS were assigned to meta-analysis; a total of 300 patients from the eligible studies were analyzed. The heterogeneity between the studies was not statistically significant (Q = 1.10; p = 0.577; I^2 = 0.00). The meta-analysis results with the fixed-effects model indicated a moderate anxiety score of 54.4%, which was not statistically significant (pooled event rate: 0.544; 95% *CI*: 0.485–0.601; p = 0.142).

Low anxiety

Three studies that evaluated low anxiety in patients with IVCS were assigned to meta-analysis; a total of 300 patients from the eligible studies were analyzed. The heterogeneity between the studies was not statistically significant (Q = 0.43; p = 0.807; I^2 = 0.00). The meta-analysis results with the fixed-effects model indicated a low anxiety score of 27.4%, which was statistically significant (pooled event rate: 0.274; 95% *CI*: 0.226–0.329; p = 0.000).

Table 3. Descriptive data of the included studies regarding patient and surgeon satisfaction

Study	Sample size	Sedation protocol	Patient satisfaction %					Surgeon satisfaction %
			comfortable	neither comfortable nor uncomfortable	slightly uncomfortable	unpleasant	traumatic	
McCrea[2] 2015	173	midazolam (the titration method, no fixed dose)	–	–	–	–	–	100.0
Gonzáles-Lemmonier et al.[4] 2010	90	midazolam (0.05 mg/kg) + fentanyl (1 µg/kg)	23.3	28.9	36.7	10.0	0	87.8
Bovaira et al.[6] 2017	180	midazolam (0.05 mg/kg) + fentanyl (1 mg/kg)	34.4	26.7	29.4	7.8	1.7	90.0
Kaviani and Ghoreishain[12] 2014	group A: 15 group B: 15	group A: midazolam (1 mg) + fentanyl (50 µg) group B: midazolam (1 mg) + ketamin (50 mg)	group A: 80.0 group B: 73.3	group A: 20.0 group B: 26.7	–	–	–	group A: 100.0 group B: 86.7
Juodzbalys et al.[13] 2005	group A: 67 group B: 20	group A: midazolam (0.1 mg/kg) + ketorolac (60 mg) group B: articaine (4%) + epinehrine	group A: 97.0 group B: 0	group A: 3.0 group B: 20.0	group A: 0 group B: 70.0	group A: 0 group B: 10.0	–	–

Surgeon and patient satisfaction

In the eligible studies, patient satisfaction was reported as comfortable, neither comfortable nor uncomfortable, slightly uncomfortable, unpleasant, or traumatic. Surgeon satisfaction was evaluated as well. The descriptive data of the related included studies is shown in Table 3. Table 4, as well as Fig. 3 and Fig. 4 present the pooled adjusted estimates of the association between IVCS and patient and surgeon satisfaction, respectively.

Patient satisfaction

Comfortable

The 4 included studies[4,6,12,13] were statistically heterogenic (Q = 47.57; p = 0.000; I^2 = 93.69). According to the pooled adjusted estimates (Fig. 3), the meta-analysis results with the random-effects model indicated that 63.3% of the patients were comfortable during surgery, which was not statistically significant (pooled event rate: 0.633; 95% *CI*: 0.322–0.862; p = 0.407).

Neither comfortable nor uncomfortable

The 4 included studies[4,6,12,13] were statistically heterogenic (Q = 12.10; p = 0.007; I^2 = 75.21). According to the pooled adjusted estimates (Fig. 3), the meta-analysis results with the random-effects model indicated that 19.8% of the patients were neither comfortable nor uncomfortable during surgery, which was statistically significant (pooled event rate: 0.198; 95% *CI*: 0.112–0.326; p = 0.000).

Slightly uncomfortable

The 4 included studies[4,6,12,13] were statistically heterogenic (Q = 13.29; p = 0.004; I^2 = 77.42). According to the pooled adjusted estimates (Fig. 3), the meta-analysis results with the random-effects model indicated that 23.0% of the patients were slightly uncomfortable during surgery, which was statistically significant (pooled event rate: 0.230; 95% *CI*: 0.117–0.402; p = 0.004).

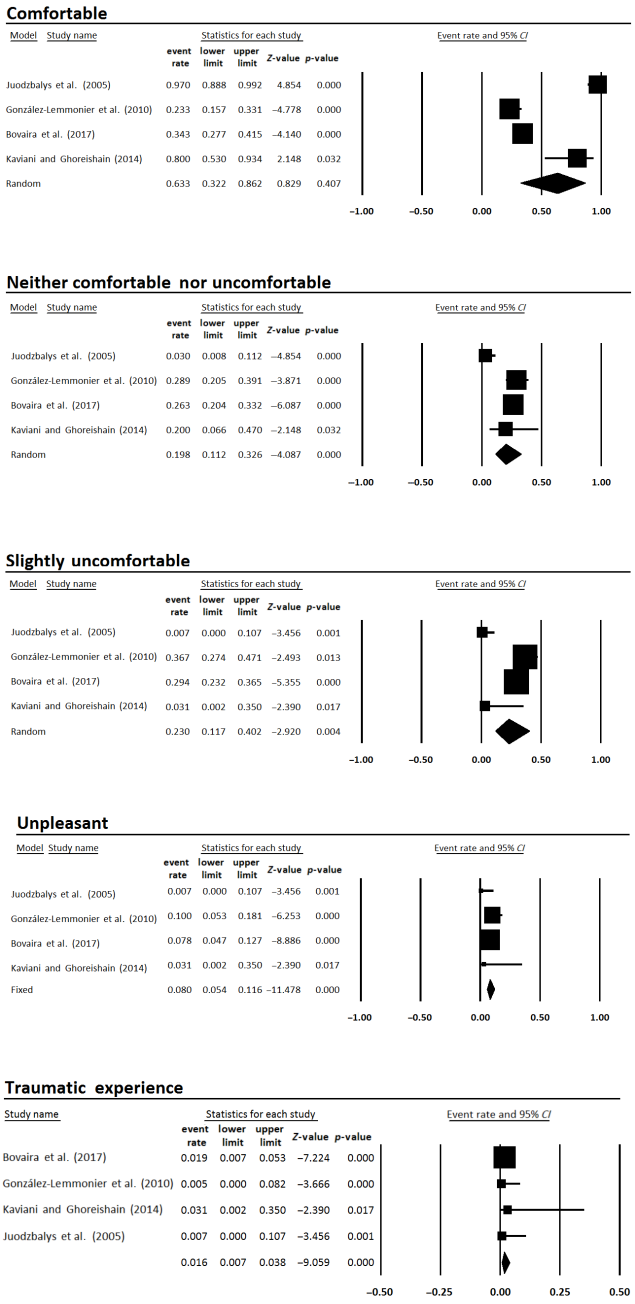

Fig. 3. Pooled adjusted estimates of the association between intravenous conscious sedation (IVCS) and patient satisfaction

Table 4. Pooled adjusted estimates of the association between intravenous conscious sedation (IVCS) and patient and surgeon satisfaction

Satisfaction	Number of studies	Effect size and 95% *CI*			Null-hypothesis two-tailed test		Heterogeneity			
		point estimate	lower limit	upper limit	*Z*-value	*p*-value	*Q*-value	df (*Q*)	*p*-value	I^2
Comfortable	4	0.633	0.322	0.862	0.829	0.407	47.57	3	0.000*	93.69
Neither comfortable nor uncomfortable	4	0.198	0.112	0.326	–4.087	0.000*	12.10	3	0.007*	75.21
Slightly uncomfortable	4	0.230	0.117	0.402	–2.920	0.004*	13.29	3	0.004*	77.42
Unpleasant	4	0.080	0.054	0.116	–11.478	0.000*	3.98	3	0.263	24.68
Traumatic experience	4	0.016	0.007	0.038	–9.059	0.000*	1.22	3	0.749	0.00
Surgeon satisfaction	4	0.922	0.833	0.965	5.635	0.000*	8.27	3	0.041*	63.73

* statistically significant.

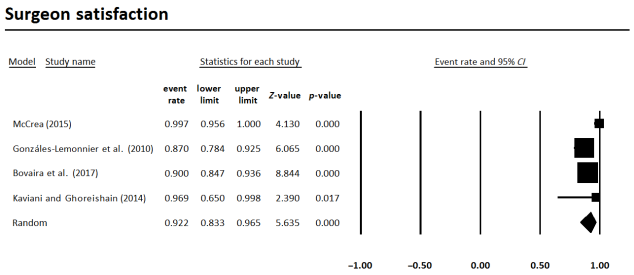

Fig. 4. Pooled adjusted estimates of the association between intravenous conscious sedation (IVCS) and surgeon satisfaction

Unpleasant

The 4 included studies[4,6,12,13] were not statistically heterogenic (Q = 3.98; p = 0.263; I^2 = 24.68). According to the pooled adjusted estimates (Fig. 3), the meta-analysis results with the fixed-effects model indicated that 8.0% of the patients felt unpleasant during surgery, which was statistically significant (pooled event rate: 0.080; 95% *CI*: 0.054–0.116; p = 0.000).

Traumatic experience

The 4 included studies[4,6,12,13] were not statistically heterogenic (Q = 1.22; p = 0.749; I^2 = 0.00). According to the pooled adjusted estimates (Fig. 3), the meta-analysis results indicated that 1.6% of the patients felt their experience was traumatic, which was statistically significant (pooled event rate: 0.016; 95% *CI*: 0.007–0.038; p = 0.000).

Surgeon satisfaction

The 4 included studies[2,4,6,12] were statistically heterogenic (Q = 8.27; p = 0.041; I^2 = 63.73). According to the pooled adjusted estimates (Fig. 4), the meta-analysis results with the random-effects model indicated that the surgeon satisfaction rate with IVCS was 92.2%, which was statistically significant (pooled event rate: 0.922; 95% *CI*: 0.833–0.965; p = 0.000).

Discussion

Like every dental surgery, dental implant surgery has the potential to cause pain, anxiety, systemic sequelae, and even life-threatening situations.[14] All of these factors may affect patient and surgeon satisfaction, surgical outcome, and even the duration of the healing period.[15,16] Anxiety control during and before dental procedures is essential to ensure the safety of the procedure, and to promote overall patient and surgeon satisfaction.[4,5] Focusing on this objective, psychological or pharmacological techniques are frequently used in the dental office,[7] especially in patients undergoing oral surgery, including dental implant surgery.[2] Digital photographs and wrapped cone-beam computed tomography (CBCT) photographs can be used to plan a more precise surgery with shorter duration, resulting in fewer postoperative complications and greater patient satisfaction.[17] Another technique is the use of sedation, which has emerged as a popular topic in the literature.[4,5,18]

Depending on the duration and difficulty of the surgery, different sedation techniques, such as inhalation or intravenous sedation, can be selected.[19] According to the results of this systematic review and meta-analysis, surgeons were highly satisfied with IVCS in dental implant surgeries (92.2%). Bovaira et al.[6] and González-Lemonnier et al.[4] used a combination of midazolam and fentanyl for IVCS. Kaviani and Goreishain compared midazolam/fentanyl with midazolam/ketamine.[12] McCrea used midazolam alone.[2] The surgeon satisfaction rate with midazolam only and midazolam/fentanyl regimes for IVCS was 100%. Midazolam/ketamine was associated with the lowest surgeon satisfaction rate.

From the patient satisfaction point of view, midazolam/fentanyl was associated with the highest degree of satisfaction.

Patient anxiety was assessed and meta-analyzed based on the data extracted from 4 studies.[2,4,6,12] Low anxiety with regard to IVCS was associated with the use of midazolam/ketamine, whereas high anxiety was reported with the use of midazolam only.

Bovaira et al. identified a significant negative relationship between the level of preoperative anxiety and patient satisfaction – the greater the preoperative anxiety, the lower the postoperative satisfaction.[6] However, the preoperative anxiety had no influence on surgeon satisfaction. A higher number of women found the experience agreeable, experiencing lower levels of preoperative anxiety as well, as compared to men. The surgeon reported an adequate level of satisfaction with the anesthesia, while most patients remained relaxed, collaborative and calm, knowing that a specialist was overseeing the surgical and sedation procedures, and understanding any possible complications that might arise.[6]

According to González-Lemonnier et al., 72.2% of patients showed moderate and high anxiety.[4] They reported a significant negative relationship between anxiety and patient satisfaction; however, patient anxiety did not affect surgeon satisfaction. In contrast with Bovaira et al.,[6] González-Lemonnier et al. reported higher anxiety in women as compared to men.[4]

Kaviani and Goreishain reported that even in low doses, both the midazolam/fentanyl and midazolam/ketamine regimes ensured proper working conditions, appropriate comfort for the patient, adequate patient and surgeon satisfaction, and short recovery time.[12] In a study by McCrea, 44% of patients declared they

experienced high anxiety and fear before surgery.[2] However, this anxiety did not affect surgeon satisfaction. The surgeon was satisfied in 100% of cases, and 99.40% of patients declared that they would prefer to use IVCS during their next implant surgery.[2] The results of the present study also indicated that the use of IVCS resulted in better working conditions, as well as better patient and surgeon satisfaction in implant surgery.

Conclusions

After a thorough review of the final articles extracted based on the study protocol, it can be concluded that the use of IVCS for implant surgery reduces patient anxiety, and also increases patient and surgeon satisfaction.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Reza Pourabbas https://orcid.org/0000-0002-8860-4006
Nazgol Ghahramani https://orcid.org/0000-0001-6399-1401
Mehrnoosh Sadighi https://orcid.org/0000-0002-7949-7237
Fatemeh Pournaghi Azar https://orcid.org/0000-0003-3492-8053
Morteza Ghojazadeh https://orcid.org/0000-0002-9946-9452

References

1. Craig DC, Boyle CA, Fleming GJ, Palmer P. A sedation technique for implant and periodontal surgery. *J Clin Periodontol*. 2000;27(12):955–959. doi:10.1034/j.1600-051x.2000.027012955.x
2. McCrea SJ. Intravenous sedation as an adjunct to advanced comprehensive dental implantology: The patient's perspective and operator satisfaction. *Br Dent J*. 2015;218(5):E11. doi:10.1038/sj.bdj.2015.192
3. Bennett C. Conscious sedation: An alternative to general anesthesia. *J Dent Res*. 1984;63(6):832–833. doi:10.1177/00220345840630060201
4. González-Lemonnier S, Bovaira-Forner M, Peñarrocha-Diago M, Peñarrocha-Oltra D. Relationship between preoperative anxiety and postoperative satisfaction in dental implant surgery with intravenous conscious sedation. *Med Oral Patol Oral Cir Bucal*. 2010;15(2):e379–e382. doi:10.4317/medoral.15.e379
5. Braidy HF, Singh P, Ziccardi VB. Safety of deep sedation in an urban oral and maxillofacial surgery training program. *J Oral Maxillofac Surg*. 2011;69(8):2112–2119. doi:10.1016/j.joms.2011.04.017
6. Bovaira M, Babiloni AH, Jovaní M, Peñarrocha-Diago M, González-Lemonnier S, Peñarrocha-Oltra D. Preoperative anxiety and its influence on patient and surgeon satisfaction in patients receiving dental implant surgeries performed under intravenous conscious sedation. *Int J Oral Maxillofac Implants*. 2017;32(4):912–918. doi:10.11607/jomi.5712
7. Seto M, Sakamoto Y, Takahashi H, Kita R, Kikuta T. Does planned intravenous sedation affect preoperative anxiety in patients?. *Int J Oral Maxillofac Surg*. 2013;42(4):497–501. doi:10.1016/j.ijom.2012.09.014
8. Khan K, Kunz R, Kleijnen J, Antes G. *Systematic Reviews to Support Evidence-Based Medicine*. 2nd ed. London, UK: CRC Press; 2011:9.
9. Higgins JP, Altman DG, GøtzschePC, et al.; Cochrane Bias Methods Group, Cochrane Statistical Methods Group. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ*. 2011;343:d5928. doi:10.1136/bmj.d5928
10. Moher D, Altman DG, Liberati A, Tetzlaff J. PRISMA statement. *Epidemiology*. 2011;22(1):128. doi:10.1097/EDE.0b013e3181fe7825
11. Owens WD, Felts JA, Spitznagel EL Jr. ASA physical status classifications: A study of consistency of ratings. *Anesthesiology*. 1978;49(4):239–243. doi:10.1097/00000542-197810000-00003
12. Kaviani N, Ghoreishain SM. Comparison of two low-dose sedation procedures on anxiety control in dental implant surgeries [in Persian]. *J Isfahan Med Sch*. 2014;32(287):768–775. http://jims.mui.ac.ir/index.php/jims/article/view/3422. Accessed November 12, 2020.
13. Juodzbalys G, Giedraitis R, Machiulskiene V, Huys LW, Kubilius R. New method of sedation in oral surgery. *J Oral Implantol*. 2005;31(6):304–308. doi:10.1563/0-771.1
14. Porter JA, von Fraunhofer JA. Success or failure of dental implants? A literature review with treatment considerations. *Gen Dent*. 2005;53(6):423–432, quiz 433,446. PMID:16366052.
15. Hawkinson RT. Dental implant failed fastener recovery systems, devices and methods. Google Patents; 1999. https://patents.google.com/patent/CA2103165A1. Accessed November 12, 2020.
16. Wismeijer D, Van Waas MA, Vermeeren JI, Muldel J, Kalk W. Patient satisfaction with implant-supported mandibular overdentures: A comparison of three treatment strategies with ITI-dental implants. *Int J Oral Maxillofac Surg*. 1997;26(4):263–267. doi:10.1016/s0901-5027(97)80864-8
17. Coolidge T, Irwin SP, Leyster KA, Milgrom P. Determinants of receiving intravenous sedation in a sample of dentally-fearful patients in the USA. *SAAD Dig*. 2012;28:52–60. PMID:23264704. PMCID:PMC3526014.
18. Jevtović-Todorović V, Todorovć S, Mennerick S, et al. Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. *Nat Med*. 1998;4(4):460–463. doi:10.1038/nm0498-460
19. Dionne RA, Yagiela JA, Moore PA, Gonty A, Zuniga J, Beirne OR. Comparing efficacy and safety of four intravenous sedation regimens in dental outpatients. *J Am Den Assoc*. 2001;132(6):740–751. doi:10.14219/jada.archive.2001.0271

Dental

and Medical Problems