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Impact of COVID-19 on the future career plans of dentists

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Abstract

Background. It is important to complement the scientific literature with the data regarding the impact of the coronavirus disease 2019 (COVID-19) pandemic on the social, work and health practices as well as on the daily life of dentists, and to promote adequate public policies.

Objectives. The aim of the study was to explore the impact of the COVID-19 pandemic on the career plans of dentists in Colombia.

Material and methods. As part of global research on the impact of COVID-19 on dentistry, a cross-sectional survey was administered via digital media to dentists in Colombia. The validated questionnaire included inquiries regarding sociodemographic variables, the perception of the risk of contagion with COVID-19 and the impact of COVID-19 on the dentists' career plans. The variables were summarized in absolute and relative frequencies, and a binomial logistic regression analysis was carried out to evaluate the impact of COVID-19 on career plans according to the independent variables.

Results. A total of 5,370 dentists answered the survey (women: 3,878; median age: 45 years; response rate: 16.85%). Most of the dentists (96%) believed that COVID-19 infection was a risk for them and 81.96% reported that the COVID-19 pandemic had some impact on their career plans, including reducing working hours (77.96%), retiring early (26.54%) and changing their career away from dentistry (18.15%). The regression model showed that older adults ($OR = 1.62$; 95% CI : 1.16, 2.26), general practitioners ($OR = 1.21$; 95% CI : 1.02, 1.44), private practice owners ($OR = 2.33$; 95% CI : 1.94, 2.79), private practice associates ($OR = 2.20$; 95% CI : 1.84, 2.63), and those with 'very probable' risk perception ($OR = 4.29$; 95% CI : 1.35, 13.60) had a significantly greater chance of the pandemic having an impact on their future career plans.

Conclusions. The pandemic has had a great impact on dentists' career plans. Dentists who are most fearful of the risk of contagion, those who are older and those who have their private practice are thinking about reducing working hours, retiring early or changing their career.

Keywords: COVID-19, dentists, career choice, social perception, SARS-CoV-2

Introduction

Coronavirus disease 2019 (COVID-19) is a viral respiratory infection evoked by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that is spread through direct contact with the surfaces infected with fomites, droplets or aerosols.¹ Airborne transmission via aerosol-generating activities has also been suggested.² New evidence has shown that the principal mode of COVID-19 transmission is via the inhalation of droplets/aerosols from the infected source rather than through the contaminated surfaces.³ COVID-19 is a highly contagious disease that can have a severe course or even cause death. Due to the rapid spread of the virus around the world, the World Health Organization (WHO) declared COVID-19 a pandemic in March 2020, generating lockdowns and restriction policies that have brought economic and political instability as well as social devastation in many countries.⁴ The risk of contagion with COVID-19 among dental healthcare workers (DHCWs) is having a tremendous impact on dentistry around the world. Fontana et al. found that DHCWs were vulnerable to the threat of COVID-19 and its psychological impact to the same extent as other essential workers.⁵

The dental profession is considered high-risk due to contact with the potentially contaminated saliva or blood carrying diverse pathogens, such as the hepatitis B virus (HBV), the hepatitis C virus (HCV) and the human immunodeficiency virus (HIV) responsible for acquired immunodeficiency syndrome (AIDS), as a result of occupational accidents.⁶ Contagion with SARS-CoV-2 through direct contact with the infected patient while performing dental procedures is possible. In addition, using a high-speed handpiece and water may generate aerosols, and result in contagion with COVID-19.⁷ Dentists' anxiety and fear of possible contagion, the governmental restrictions regarding elective dental procedures, and lockdown isolation have created a financial impact and uncertainty in the context of dentists' future career plans.^{8,9} Such fear can cause mental health problems and post-traumatic stress disorder symptoms, which may change future career objectives in terms of work patterns or retirement plans among DHCWs.¹⁰

Dentistry is a liberal profession that entails particular employment conditions, practice settings, remuneration, and social status in each country, and the career prospects may also vary.^{11,12} Lo Sasso et al. found variations in job satisfaction among dentists with different practice settings (individual practice, small-group practice or large-group practice).¹³ On the other hand, Campus et al. conducted a study on the promotion and development of young professionals in Switzerland.¹⁴ They found that a significantly low percentage of dentists held a negative view of their professional future; the majority of young dentists were satisfied with their career prospects.¹⁴ Janulyte et al. found that Lithuanian general dentists and dental specialists

planned to retire beyond the official retirement age or to emigrate to work in other countries.¹⁵ Gallagher et al. found that among final-year dental students at King's College of London, UK, the factors influencing their long-term career plans were related to personal considerations, such as the standard of living, work-life balance and financial security.¹⁶ Nashleanas et al. observed that although the majority of dental students in the United States hoped to pursue specialty training after dental school, their high educational debts made them choose to practice general dentistry immediately after graduation.¹⁷

To the best of our knowledge, no studies on changes in dentists' career plans due to the COVID-19 pandemic are available in the literature. Such information is important for implementing adequate public policies; it may also alert dental associations and dental schools of the potential problems the COVID-19 pandemic can bring to the dental profession so that appropriate actions could be undertaken to avoid negative consequences to the oral health of the population. Therefore, the aim of the current study was to explore the impact of the COVID-19 pandemic on the career plans of Colombian dentists.

Methods

This cross-sectional study was based on the data collected from an anonymous survey on the impact of the COVID-19 pandemic on dentistry in Colombia. The protocol of the study was approved by the Ethics Committee of Fundación Universitaria CIEO – UniCIEO, Bogota, Colombia (No. 101, file 62). All respondents completed an informed consent form that was embedded on the 1st page of the questionnaire; participation in the study was completely voluntary. The study fully complied with the World Medical Association (WMA) Declaration of Helsinki, and reporting followed the STROBE (STrengthening the Reporting of OBServational studies in Epidemiology) guidelines.

The questionnaire was part of a global survey conducted by the Collaborative Group for COVID-19, an organization to which the authors of this study are affiliated.¹⁸ The Colombian version of the questionnaire had 31 questions, 21 from the global core questionnaire and 10 additional questions on the impact of COVID-19 on future career planning, teledentistry and the management of ventilation systems (the questionnaire is available from the corresponding author on reasonable request). As some of the questions were originally in English, a systematic approach to translation and adaptation was implemented. It consisted of 5 steps: forward translation; expert panel discussion; backward translation; a pre-test; cognitive briefing; and consensus on the final version. The survey was validated in terms of appearance, content validity and reproducibility in a pilot test. Thirty dentists who work in Colombia were recruited using simple random

sampling and invited to participate in the pilot test. Of these, 10 were asked to assess face validity (“Does the test ‘look like’ a measure of the construct of interest?”) and semantic comprehension, and the remaining 20 were asked to assess test–retest reliability (the repeated application of the instrument at 4–7-day intervals).

The sample was collected by convenience sampling from the initial population of 31,872 dentists certified to work in Colombia in 2019.¹⁹ Using the OpenEpi software, v. 3.01 (<https://www.openepi.com>) on the data from the pilot study, the required sample size of 2,341 subjects was obtained. A 99.9% confidence level was considered, and an estimated prevalence of 70% for the impact of COVID-19 on future career planning was given to establish an estimate within $\pm 3\%$ of this value. The inclusion criteria were dentists working in Colombia. Surveys with errors in the information record were excluded.

The self-administered online questionnaire was distributed from June 19 to July 24, 2020. The survey link was distributed through digital media (Facebook groups, WhatsApp messages, e-mails, and institutional invitations) to several dental schools and dental associations in Colombia. The Google Forms settings were adjusted to disallow missing or incomplete answers, and modified to remove access to the link after a response was submitted. To control information bias, in the institutional databases, the questionnaire was forwarded by e-mail only to the dentists who had not previously opened the mail. Additionally, in the introduction and the final part of the survey, a warning sign reminded the respondents not to fill out the questionnaire again if it had already been submitted. No incentives were offered to the participants.

The following independent variables were assessed: demographics – sex, age, and work locality; specialty and practice setting; self-reported COVID-19 testing and contagion; the perception of confidence and of the risk of contagion; and the implementation of preventive measures in the practice (4 categories: DHCW measures, disinfection, patient measures, dental office measures) and the use of personal protective equipment (PPE). The preventive measures and the elements of PPE are detailed in Table 1. Age was determined as a continuous variable; however, for the statistical analysis, it was re-categorized into 4 age groups: young adult (22–35 years); adult (36–45 years); mature adult (46–59 years); and older adult (60 years and above). The work locality variable was categorized using the CEPAL (Economic Commission for Latin America and the Caribbean) classification according to the territorial agglomeration index: urban provinces (97–673 inhabitants per km²); intermediate provinces (15–277 inhabitants per km²); and rural provinces (0–127 inhabitants per km²).²⁰

The dependent variable was ‘the impact of the COVID-19 pandemic on career plans’. This variable offered 6 multiple-choice options: consider retiring early; reduce working hours; move from the current practice location; change the career away from dentistry; move to

a non-clinical dental position; and no impact on career plans. For the statistical analysis, this variable was dichotomized into ‘impact’ and ‘no impact’.

Statistical analysis

The data was collected in an Excel file provided by Google Forms. Anonymous data analyses were performed by one of the researchers (S.P.P.R), and checked for consistency and quality. The statistical analysis was performed using Stata Statistical Software: Release 16 (StataCorp, College Station, USA). The variables were summarized in absolute and relative frequencies. Additionally, a binomial logistic regression analysis was carried out to evaluate the probability of the impact of the COVID-19 pandemic on career plans according to the independent variables. Variables with a p -value ≤ 0.20 in the bivariate analysis were included in the logistic regression model (the backward stepwise procedure). The quality of the model adjustments was analyzed with the log of the likelihood and the Akaike information criterion (AIC). The model with the lowest values of the likelihood ratio (LR) and AIC was selected. The significance level was set at 5%.

Results

Test–retest reliability was high, with a kappa coefficient ranging from 0.91 to 0.96 (confidence interval (CI): 0.77, 0.96). In total, 5,375 completed surveys were obtained; 5 were discarded due to errors in the information record, leaving 5,370 viable surveys. With a total of 31,872 dentists registered to work in the Colombian territory, the corresponding response rate of the survey was 16.85%.

The descriptive statistics of the sample are presented in Table 2. Women accounted for 72.22% of the sample, and almost half of the respondents were above the age of 45. More than 80% worked in urban provinces (81.71%), more than 90% were general practitioners (41.94%) or clinical specialists (48.21%), and 80.58% worked in a private practice (as an owner or an associate). At the time of conducting the survey, 8.53% of the respondents had been tested for COVID-19 and 0.61% were COVID-19 positive. Fully 96% of the respondents believed that COVID-19 infection was a risk for dentists and 58.98% of them believed that the risk of COVID-19 transmission in the dental practice was higher than the risk associated with going to a supermarket. Of the 81.96% respondents who reported that the COVID-19 pandemic had some impact on their career plans, 26.54% considered retiring early, 77.96% thought about reducing working hours, 9.27% about moving from the current practice location, 18.15% about changing their career away from dentistry, and 16.02% considered moving to a non-clinical dental position. Adherence to biosecurity procedures was between 97.12% and 99.21%, and the frequency of the use of some form of PPE was 100%.

Table 1. Categories of preventive measures against coronavirus disease 2019 (COVID-19) infection

Category	Preventive measures
DHCW measures	taking the temperature of all staff and excluding those with a temperature above 37.5°C handwashing for the clinical staff before and after each procedure
Disinfection	disinfecting surfaces with 70% alcohol disinfecting surfaces with 0.5% sodium hypochlorite other common clinical disinfectants disinfecting surfaces several times a day (bells, door handles, POS credit card readers, the waiting room, and the reception) disinfecting all elements and surfaces in the clinical area several times a day removing all disposable protective items and disinfecting non-disposable protective items
Patient measures	checking the patient's state of health before giving an appointment postponing appointments for people who are elderly or have systemic pathologies taking the patient's temperature on arrival hand washing/sanitizer gel for patients before entering the clinical area maintaining at least a 1-meter distance between patients offering the patient a mask while they are waiting disposable shoe covers over the patient's shoes preoperative mouthwash with 1% hydrogen peroxide preoperative mouthwash with 0.12–0.20% chlorhexidine preoperative mouthwash with 0.20–1.00% povidone iodine preoperative mouthwash with 0.05-0.10% cetylpyridinium chloride using a dilution of mouthwash
Dental office measures	phone triage spacing appointments erecting protective dividing screens ventilating the clinical area and the waiting room frequently ventilating the clinical area for at least 10 min between patients removing magazines and books from the waiting area leaving coats, bags or other personal objects outside the clinical area using rotary instruments with a non-return valve
PPE	surgical mask FFP2, FFP3 or N95 mask silicone respirator disposable scrub cap non-disposable fluid-resistant coat disposable fluid-resistant coat sterile disposable fluid-resistant coat protective overall protective glasses or faceshield safety glasses or visor sterile disposable gloves disposable shoe covers rubber dam checking the patient's state of health before giving an appointment

DHCW – dental healthcare worker; PPE – personal protective equipment; POS – point of sale.

Table 2. Sociodemographic characteristics and the questionnaire answers ($N = 5,370$ unless marked otherwise)

Variable		Absolute frequency (<i>n</i>)		Relative frequency (%)		
sex ($N = 5,369$; other: $n = 1$)	male	1,491		27.77		
	female	3,878		72.22		
age (categorized)	young adult (22–35 years)	1,341		24.97		
	adult (36–45 years)	1,488		27.71		
	mature adult (46–59 years)	2,111		39.31		
	older adult (≥ 60 years)	430		8.01		
work locality	urban	4,388		81.71		
	intermediate	510		9.50		
	rural	466		8.68		
	NA – unemployed	6		0.11		
specialty	general practitioner	2,252		41.94		
	clinical specialist	2,589		48.21		
	public health	470		8.75		
	other	59		1.10		
practice setting	–	yes	no	yes	no	
	private practice (owner)	2,401	2,969	44.71	55.29	
	private practice (tenant/associate)	1,926	3,444	35.87	64.13	
	public or private sector salaried employee	1,637	3,733	30.48	69.52	
	academic/research worker	723	4,647	13.46	86.54	
Categorical variables	Have you ever been tested for COVID-19?	administrative worker	372	4,998	6.93	93.07
		yes	458		8.53	
	What was the result of your COVID-19 test?	no	4,912		91.47	
		COVID-19 positive	33		0.61	
		COVID-19 negative	425		7.91	
	Have you undertaken any course (with/without certification) on COVID-19?	NA – I have not been tested	4,912		91.47	
		yes	3,498		65.14	
	Do you think that you know enough about COVID-19?	no	1,872		34.86	
		yes	3,699		68.88	
	Do you believe that there is a real risk for a dentist of contracting COVID-19 at work?	no	1,671		31.12	
very likely		3,719		69.26		
likely		1,431		26.65		
unlikely		207		3.85		
Do you believe that the risk of COVID-19 transmission in the dental practice is	very unlikely	13		0.24		
	higher than the risk associated with going to a supermarket?	3,167		58.98		
	comparable to the risk associated with going to a supermarket?	732		13.63		
How sure are you that you can avoid becoming infected with COVID-19 whilst working?	lower than the risk associated with going to a supermarket?	1,471		27.39		
	very confident	493		9.18		
	reasonably confident	2,961		55.14		
	not so confident	1,603		29.85		
How has the COVID-19 pandemic influenced your career plans (dichotomous)	not at all confident	313		5.83		
	impact on career plans	4,401		81.96		
	no impact on career plans	969		18.04		

		Variable	Absolute frequency (n)		Relative frequency (%)	
			yes	no	yes	no
Categorical variables	How has the COVID-19 pandemic influenced your career plans (multiple options)	–				
		consider retiring early (N = 4,401)	1,168	3,233	26.54	73.46
		reduce working hours (N = 4,401)	3,431	970	77.96	22.04
		move from the current practice location (N = 4,401)	408	3,993	9.27	90.73
		change the career away from dentistry (N = 4,401)	799	3,602	18.15	81.85
		move to a non-clinical dental position (N = 4,401)	705	3,696	16.02	83.98
		no impact on career plans	969	4,401	18.04	81.96
	DHCW measures (N = 4,168)	yes	4,048		97.12	
		no	120		2.88	
	disinfection (N = 4,168)	yes	4,092		98.18	
		no	76		1.82	
patient measures (N = 4,168)	yes	4,135		99.21		
	no	33		0.79		
dental office measures (N = 4,168)	yes	4,135		99.21		
	no	33		0.79		
PPE (N = 4,282)	yes	4,282		100.00		
	no	0		0.00		
Continuous variable	age [years] Me (min–max)		45 (22–82)			

NA – not applicable; COVID-19 – coronavirus disease 2019; Me – median; min – minimum; max – maximum.

Note: The questions on the implementation of preventive measures and PPE were answered only by the dentists in clinical practice, working under the conditions of isolation during the COVID-19 pandemic.

The bivariate analysis results are shown in Table 3. Evidence was found ($p < 0.05$) of associations between the impact of the COVID-19 pandemic on career plans and age group, specialty, practice setting, COVID-19 testing, the probability of contagion, and confidence with regard to contagion. Higher frequencies of impact were observed in older adults (87.44%), general practitioners (83.26%), private practice owners (86.34%), non-tested dentists (82.31%), dentists who thought that contagion was very probable (83.25%), and those who were not very confident that they could avoid being infected (84.59%).

The logistic regression model (Table 4) provides the odds ratio (OR) of significant variables to predict the chance of the COVID-19 pandemic having an impact on future career plans (yes/no) and a 95% CI for the OR. The final model showed that mature adults (OR = 1.23; 95% CI: 1.01, 1.48), older adults (OR = 1.62; 95% CI: 1.16, 2.26), general practitioners (OR = 1.21; 95% CI: 1.02, 1.44), private practice owners (OR = 2.33; 95% CI: 1.94, 2.79), private practice associates (OR = 2.20; 95% CI: 1.84, 2.63), and those with 'very probable' risk perception (OR = 4.29;

95% CI: 1.35, 13.60) had a significantly greater chance of the pandemic having an impact on their future career plans ($p < 0.05$). Meanwhile, for those in academic/research (OR = 0.73; 95% CI: 0.59, 0.90) and administrative positions (OR = 0.62; 95% CI: 0.47, 0.83), there was a significantly lower chance of the disease impacting their future career plans ($p < 0.05$).

Discussion

The ongoing changes in the dentistry field caused by the COVID-19 pandemic are a challenge for private practices, public dental healthcare systems, dental schools,^{11,21,22} scientific associations, and decision-makers responsible for health policies to overcome the potential negative impact of the current pandemic on the future career plans of dentists around the world.^{11,21–23} The results of the present survey provide relevant information about the possible factors associated with the impact of the COVID-19 pandemic on the career plans of dentists in Colombia.

Table 3. Bivariate analysis of the impact of the COVID-19 pandemic on dentists' career plans (N = 5,370 unless marked otherwise)

Categorical variable		How has the COVID-19 pandemic influenced your career plans?				p-value
		impact (N = 4,401)		no impact (N = 969)		
Sex (N = 5,369; other: n = 1)	male	1,209 (81.09)		282 (18.91)		0.296
	female	3,192 (82.31)		686 (17.69)		
Age (categorized)	young adult (22–35 years)	1,066 (79.49)		275 (20.51)		0.001*
	adult (36–45 years)	1,210 (81.32)		278 (18.68)		
	mature adult (46–59 years)	1,749 (82.85)		362 (17.15)		
	older adult (≥60 years)	376 (87.44)		54 (12.56)		
Work locality	urban	3,600 (82.04)		788 (17.96)		0.909
	intermediate	415 (81.37)		95 (18.63)		
	rural	380 (81.55)		86 (18.45)		
Specialty	general practitioner	1,875 (83.26)		377 (16.74)		<0.001*
	clinical specialist	2,147 (82.93)		442 (17.07)		
	public health	333 (70.85)		137 (29.15)		
	other	46 (77.97)		13 (22.03)		
Practice setting	–	yes	no	yes	no	–
	private practice (owner)	2,073 (86.34)	2,328 (78.41)	328 (13.66)	641 (21.59)	<0.001*
	private practice (tenant/associate)	1,649 (85.62)	2,752 (79.91)	277 (14.38)	692 (20.09)	<0.001*
	public or private sector salaried employee	1,264 (77.21)	3,137 (84.03)	373 (22.79)	596 (15.97)	<0.001*
	academic/research worker	563 (77.87)	3,838 (82.59)	160 (22.13)	809 (17.41)	0.002*
	administrative worker	248 (66.67)	4,153 (83.09)	124 (33.33)	845 (16.91)	<0.001*
Have you ever been tested for COVID-19?	yes	358 (78.17)		100 (21.83)		0.027*
	no	4,043 (82.31)		869 (17.69)		
What was the result of your COVID-19 test?	COVID-19 positive	28 (84.85)		5 (15.15)		0.079
	COVID-19 negative	330 (77.65)		95 (22.35)		
	NA – I have not been tested	4,043 (82.31)		869 (17.69)		
Have you undertaken any course (with/without certification) on COVID-19?	yes	2,859 (81.73)		639 (18.27)		0.562
	no	1,542 (82.37)		330 (17.63)		
Do you think that you know enough about COVID-19?	yes	3,013 (81.45)		686 (18.55)		0.156
	no	1,388 (83.06)		283 (16.94)		
Do you believe that there is a real risk for a dentist of contracting COVID-19 at work?	very likely	3,096 (83.25)		623 (16.75)		<0.001*
	likely	1,151 (80.43)		280 (19.57)		
	unlikely	146 (70.53)		61 (29.47)		
	very unlikely	8 (61.54)		5 (38.46)		
Do you believe that the risk of COVID-19 transmission in the dental practice is	higher than the risk associated with going to a supermarket?	2,627 (82.95)		540 (17.05)		0.066
	comparable to the risk associated with going to a supermarket?	585 (79.92)		147 (20.08)		
	lower than the risk associated with going to a supermarket?	1,189 (80.83)		282 (19.17)		
How sure are you that you can avoid becoming infected with COVID-19 whilst working?	very confident	382 (77.48)		111 (22.52)		0.002*
	reasonably confident	2,406 (81.26)		555 (18.74)		
	not so confident	1,356 (84.59)		247 (15.41)		
	not at all confident	257 (82.11)		56 (17.89)		
DHCW measures	yes	3,344 (82.61)		704 (17.39)		0.072
	no	98 (81.67)		22 (18.33)		
Disinfection	yes	3,383 (82.67)		709 (17.33)		0.251
	no	59 (77.63)		17 (22.37)		
Patient measures	yes	3,418 (82.66)		717 (17.34)		0.134
	no	24 (72.73)		9 (27.27)		
Dental office measures	yes	3,417 (82.64)		718 (17.36)		0.299
	no	25 (75.76)		8 (24.24)		
PPE	yes	3543 (82.74)		739 (17.26)		NA
	no	0 (0.00)		0 (0.00)		

Data presented as absolute (relative) frequency (n (%)). * statistically significant.

Table 4. Logistic regression model for the predictor variables for the chance of the COVID-19 pandemic having an impact on dentists' future career plans

Variable	OR crude		OR full model		OR final regression model		
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	
Sex	male	1	–	–	–	–	
	female	1.08 (0.93, 1.26)	0.296	–	–	–	
Age (categorized)	young adult (22–35 years)	1	–	1	–	1	
	adult (36–45 years)	1.12 (0.93, 1.35)	0.222	1.31 (1.05, 1.64)	0.015*	1.10 (0.90, 1.34)	0.332
	mature adult (46–59 years)	1.25 (1.05, 1.48)	0.013*	1.48 (1.19, 1.85)	<0.0001*	1.23 (1.01, 1.48)	0.030*
	older adult (≥60 years)	1.80 (1.31, 2.46)	<0.0001*	2.02 (1.34, 3.06)	0.001*	1.62 (1.16, 2.26)	0.004*
Work locality	urban	1	–	–	–	–	
	intermediate	0.95 (0.75, 1.21)	0.710	–	–	–	
	rural	0.96 (0.75, 1.23)	0.791	–	–	–	
Specialty	clinical specialist	1	–	1	–	1	
	general practitioner	1.02 (0.88, 1.19)	0.759	1.32 (1.09, 1.60)	0.005*	1.21 (1.02, 1.44)	0.028*
	public health	0.50 (0.40, 0.62)	<0.0001*	1.56 (1.04, 2.34)	0.028*	0.92 (0.69, 1.22)	0.577
	other	0.72 (0.39, 1.35)	0.320	3.15 (0.92, 10.74)	0.067	1.26 (0.66, 2.42)	0.475
Practice setting	private practice (owner)	1.74 (1.50, 2.01)	<0.0001*	1.92 (1.48, 2.47)	<0.0001*	2.33 (1.94, 2.79)	<0.0001*
	private practice (tenant/associate)	1.50 (1.29, 1.74)	<0.0001*	1.94 (1.52, 2.47)	<0.0001*	2.20 (1.84, 2.63)	<0.0001*
	public or private sector salaried employee	0.64 (0.56, 0.74)	<0.0001*	0.83 (0.66, 1.04)	0.112	–	–
	academic/research worker	0.74 (0.61, 0.89)	0.002*	0.84 (0.65, 1.07)	0.166	0.73 (0.59, 0.90)	0.003*
	administrative worker	0.41 (0.32, 0.51)	<0.0001*	0.63 (0.41, 0.97)	0.035*	0.62 (0.47, 0.83)	0.001*
Have you ever been tested for COVID-19?	no	1	–	1	–	–	
	yes	0.77 (0.61, 0.97)	0.028*	0.28 (0.05, 1.44)	0.129	–	–
What was the result of your COVID-19 test?	COVID-19 negative	1	–	1	–	–	
	COVID-19 positive	1.58 (0.59, 4.21)	0.790	1.71 (0.54, 5.34)	0.355	–	–
	NA – I have not been tested	1.31 (1.03, 1.67)	0.160	0.33 (0.06, 1.69)	0.185	–	–
Have you undertaken any course (with/without certification) on COVID-19?	yes	0.96 (0.83, 1.10)	0.562	–	–	–	
	no	1	–	–	–	–	
Do you think that you know enough about COVID-19?	no	1	–	–	–	–	
	yes	0.90 (0.77, 1.04)	0.156	0.91 (0.75, 1.09)	0.305	–	–
Do you believe that there is a real risk for a dentist of contracting COVID-19 at work?	very unlikely	1	–	–	–	1	
	very likely	3.11 (1.01, 9.52)	0.047*	3.50 (1.06, 11.62)	0.040*	4.29 (1.35, 13.60)	0.013*
	likely	2.57 (0.83, 7.91)	0.100	3.23 (0.97, 10.71)	0.055	3.14 (0.98, 10.01)	0.052
	unlikely	1.50 (0.47, 4.75)	0.495	1.98 (0.58, 6.80)	0.275	1.68 (0.51, 5.54)	0.389

Variable	OR crude		OR full model		OR final regression model	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Do you believe that the risk of COVID-19 transmission in the dental practice is	comparable to the risk associated with going to a supermarket?	1	–	–	–	–
	higher than the risk associated with going to a supermarket?	1.22 (0.99, 1.49)	0.053	1.07 (0.83, 1.38)	0.611	–
	lower than the risk associated with going to a supermarket?	1.05 (0.85, 1.32)	0.611	0.91 (0.69, 1.19)	0.475	–
How sure are you that you can avoid becoming infected with COVID-19 whilst working?	not at all confident	1	–	–	–	–
	very confident	0.75 (0.52, 1.07)	0.115	0.75 (0.47, 1.20)	0.234	–
	reasonably confident	0.94 (0.70, 1.28)	0.713	1.02 (0.69, 1.53)	0.888	–
	not so confident	1.20 (0.87, 1.65)	0.271	1.37 (0.90, 2.07)	0.136	–
DHCW measures	no	1	–	–	–	–
	yes	1.06 (0.67, 1.70)	0.789	–	–	–
Disinfection	no	1	–	–	–	–
	yes	1.37 (0.80, 2.37)	0.253	–	–	–
Patient measures	no	1	–	–	–	–
	yes	1.79 (0.83, 3.87)	0.139	1.67 (0.75, 3.74)	0.143	–
Dental office measures	no	1	–	–	–	–
	yes	1.52 (0.68, 3.39)	0.303	–	–	–
PPE	no	0	–	–	–	–
	yes	1	–	–	–	–

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

The sample in the present study was composed predominantly of women (72%), which is in line with the preponderance of female dentists in Colombia (75%). Age and work localities were also representative of the population of dentists registered to work in Colombia.¹⁹

On June 19, 2020, when we began administering the survey, 63,276 people had tested positive for COVID-19 in Colombia and 2,045 deaths were reported due to the disease. By the end of the survey, on July 24, 2020, the number of cases had risen to 233,451 and 7,975 deaths.²⁴ As of July 24, 2020, there were 33 reported cases of dentists infected with COVID-19 in the country.²⁵ According to our results, the respondents self-reported that only 8.53% of them (458 dentists) had been tested for COVID-19, and of those, 7.21% (33 dentists) were COVID-19 positive. Similar results were obtained by Fontana et al., who reported 6% of the surveyed DHCWs were tested and 4% were positive for COVID-19.⁵

In the current survey, 96% of the dentists perceived COVID-19 as a real risk for dentists and 58.98% of the respondents believed that the risk of COVID-19 transmission in dental practice was higher than that of going to a supermarket. However, 64.32% (reasonably

confident or very confident) were optimists about the possibility of avoiding COVID-19 infection while working. These results suggest that even though the respondents were aware of the risk from face-to-face dental procedures, they felt safe in some way. This may be due to the perception of security afforded by strict adherence to biosecurity procedures (97.12% to 99.21%) and the use of PPE reported by 100% of those surveyed. Fontana et al. found that DHCWs were significantly less fearful than non-dental healthcare providers and non-healthcare essential workers.⁵

Regarding the impact on career plans, in the present study, 81.96% of the dentists reported that the COVID-19 pandemic had some impact on their career plans; 77.96% of the respondents considered reducing working hours, 26.54% retiring early and 34.17% leaving face-to-face patient care, either through changing the career away from dentistry (18.15%) or moving to a non-clinical dental position (16.02%). However, we did not find statistically significant differences according to sex with regard to the impact of COVID-19 on future career plans. Without a doubt, the COVID-19 pandemic has been one of the most catastrophic events in the recent history

of humanity, and it still causes uncertainty about personal and professional future.²² Sinjari et al. surveyed 440 Italian dentists in the Abruzzo region during the COVID-19 outbreak and found that 66.6% reported apprehension about their professional future.²⁶ The high percentage of dentists who considered reducing working hours or leaving face-to-face patient care could be due to diverse factors, such as higher stress they face at work, the fear of spreading the infection to their families, or the financial burden of additional PPE and biosecurity products to perform dental procedures.⁹

We also found significant differences ($p < 0.05$) showing that those whose career plans were most impacted by the COVID-19 pandemic were older adults (87.44%), general practitioners (83.26%), private practice owners (86.34%), non-tested dentists (82.31%), dentists that perceived the risk of contagion as very probable (83.25%), and those with little confidence in avoiding becoming infected in the dental practice (84.59%). Many authors have reported the aging of the dental workforce around the world.^{15,27} Our sample, which included dentists aged 22–82 years, showed that many people working in the dental healthcare system in Colombia are older. Since age is considered a risk factor for disease severity and death from COVID-19,²⁸ this could be the cause of the high number of dentists who considered retiring early. General dentists and private practice owners might be more impacted, because they may perceive that both their safety and their financial situation are compromised by the current pandemic. These findings are similar to those of Lo Sasso et al., who found that the income of the dentists working in individual private practices was lower than that of those working in small and large groups.¹³ As for our result that non-tested dentists were more affected than those who had been tested for COVID-19, this finding could be due to the fact that testing may alleviate stress and anxiety.⁵ Also, the most affected group were those who considered themselves more at risk of contagion and less confident of avoiding it. This could be explained by the state of anxiety and fear that many dentists around the world are facing, which may overwhelm them and impact their career plans.⁸

In our study, we also evaluated which factors were the best predictors for the probability of the pandemic having some impact on career plans. The regression analysis confirmed the impact of COVID-19 on dentists' career plans. Being over 60 years old ($OR = 1.62$; 95% CI : 1.16, 2.26), being a general practitioner ($OR = 1.21$; 95% CI : 1.02, 1.44), being a private practice owner ($OR = 2.33$; 95% CI : 1.94, 2.79) or associate ($OR = 2.20$; 95% CI : 1.84, 2.63), and having the perception of contagion as very probable ($OR = 4.29$; 95% CI : 1.35, 13.60) were significant risk factors for career plans being impacted by COVID-19. Dentists in academic/research ($OR = 0.73$; 95% CI : 0.59, 0.90) or administrative positions ($OR = 0.62$; 95% CI : 0.47, 0.83) had a significantly lower probability of their future career plans being impacted ($p < 0.05$).

Limitations

The limitations of the present study are the potential bias that self-administered surveys and cross-sectional studies carry, and the fact that the survey was conducted in the context of the particular situation of the country with regard to the COVID-19 outbreak at the moment the dentists answered the survey, affecting their responses about future plans. These limitations could influence the results; thus, caution is recommended in generalizing these findings. It is also important to take into account that at the time of the survey, the COVID-19 vaccine was not available globally. It would be interesting to know if vaccination has changed the perception of dentists toward their current career plans.

Conclusions

The COVID-19 pandemic has affected the career plans of dentists, with the impact being greater among older clinicians, dentists with a privately owned practice and dentists with a greater perception of the risk of contagion.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Fundación Universitaria CIEO – UniCIEO, Bogota, Colombia (No. 101, file 62). All respondents provided informed written consent for the participation in the study.

Data availability


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


Consent for publication

Not applicable.

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Gender differences in concerns, stress levels and behavior changes in dental academics in Iran during the COVID-19 pandemic: A cross-sectional study

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Conflict of interest

None declared

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Abstract

Background. Coronavirus disease 2019 (COVID-19) has placed a significant psychological burden on the healthcare personnel. Dental academics play a crucial role in the promotion of public oral health and the education of dental students.

Objectives. The aim of the study was to assess COVID-19-related concerns, stress and behavior changes in Iranian dental academics, determine the associated factors, and assess the potential gender differences.

Material and methods. In this cross-sectional study, we shared a researcher-developed questionnaire with Iranian dental academics through social media and e-mail. The questionnaire contained 4 sections: Background (8 items and additional 6 knowledge questions); Concerns (18 items); Stress (based on the 15-item Impact of Event Scale (IES)); and Behavior Change (5 items). We divided the concerns by means of the principal component analysis (PCA) into 3 various components of the fear of infection, concerns over professional responsibilities and concerns over restrictions. The backward stepwise multilevel linear and logistic regression analyses served to discover the association of other factors with stress and behavior changes.

Results. Out of 274 respondents, 66% were female, and 78% were 45 years old or younger. Approximately half of the respondents demonstrated moderate to severe stress and the median (*Me*) for behavior change was 46.5 out of 50. The greatest concern expressed by the participants referred to the fear of becoming infected by their patients and spreading the virus to their loved ones. Being female, living with parents and not having any administrative role, along with greater fear of infection, concerns over restrictions and academic experience were associated with higher levels of COVID-19-related stress. Being female and having an administrative role, along with greater fear of infection and knowledge, and more daily patient visits were associated with desirable COVID-19-related behavior changes.

Conclusions. The COVID-19 pandemic has significantly affected dental academics' psychological state, leading to various levels of concern, stress and behavior change. Supportive and educational programs must target those with high fear and stress levels to prevent undesirable behaviors.

Keywords: health behavior, psychological stress, fear, COVID-19, dental faculty

Introduction

Coronavirus disease 2019 (COVID-19) emerged in China in December 2019¹ and quickly developed into a pandemic by March 2020.² Until November 2021, about 250 million cases and 5.1 million deaths had occurred worldwide due to the pandemic.³

Many countries have imposed restrictions to control the spread of the infection.⁴ Under such circumstances, the number of people with mental health issues tends to exceed the number of people directly affected by the infection.⁵ The COVID-19 pandemic has exacerbated anxiety, depression, fear, stress, and sleep problems among the public.⁶ The highly contagious nature of the disease and its fatal consequences have also led to lifestyle changes, such as avoiding crowds, social distancing, more frequent handwashing, and wearing masks.⁷

Due to close contact with patients, healthcare professionals are at high risk of contracting the disease.^{8,9} This causes psychological problems, such as the fear of spreading the disease to their family or loved ones, and possibly post-traumatic stress disorder (PTSD).¹⁰ This psychological distress was also apparent among healthcare workers (HCWs) during the severe acute respiratory syndrome (SARS) outbreak, with an elevated risk for those more exposed,¹¹ and was associated with the fear of contagion¹² and concern about family.¹¹

Among HCWs, dentists are the highest-risk group to contract COVID-19¹³ due to exposure to droplets and aerosols, which are the main transmission routes for the virus.^{8,14} In a situation like this, even standard protective measures are relatively ineffective in preventing the transmission of the disease,⁸ thus making dentists susceptible to severe distress.¹⁵

Researchers and academics are also dealing with the psychological impact of the COVID-19 pandemic due to the challenges faced by dental and medical schools.¹⁶ The adoption of e-learning, the suspension of several research projects and the threat of unemployment may have created new additional stressors.¹⁷

A dental academic is a clinician and a researcher at the same time, and also trains students in an educational clinic, along with teaching them theoretical courses. Therefore, in the pandemic situation, dental academics need to protect themselves, their loved ones, patients, students, and the public. However, the psychological impact of the pandemic on productivity, well-being and quality of life may push people into panic and making mistakes that lead to irrational decisions and behavior.¹⁸ Mental illnesses can also negatively affect the decision-making ability of the medical staff, including dentists, leading to the suboptimal treatment of patients as well as burnout.^{19,20}

Identifying the specific sources of stress and undesirable behavior is essential for developing effective approaches to tackle these problems. It should be

the primary focus of supportive efforts,²¹ along with targeting attention at those who are in a greater need of help.

There are plenty of studies on the psychological impact of the COVID-19 pandemic among dental students and dentists; however, very few have assessed psychological outcomes in dental academics. Therefore, we aimed to assess COVID-19-related concerns, stress and behavior changes in Iranian dental academics, determine the associated factors, and assess the potential gender differences.

Material and methods

Study design

This online survey-based cross-sectional study was part of a multinational study conducted in Alexandria University, Egypt,²² and it was performed in Tehran University of Medical Sciences, Egypt, in April 2020. The research was approved by the institutional ethics committee (approval ID: IR.TUMS.DENTISTRY.REC.1399.001).

Study population and sampling

The study population consisted of all the 1,826 officially registered dental academics employed in Iran.²³ By considering the elevated distress among dental professionals to be 11.5%²⁴ and the confidence level to be 95%, we needed at least 145 participants to obtain a margin of error less than 5%.²⁵

We used convenient sampling and shared the survey link together with a brief explanation of the study with Iranian dental academics through e-mail and social media (Telegram, WhatsApp, Instagram) – privately, and also through social media groups and channels exclusive to dental academics. We also sent reminders 1 week after sending the invitation for response rate maximization. Additionally, we used the snowball method and asked the receivers to share the invitation with their fellow dental academics. The survey was available from April 8 to April 21, 2020, and an estimate of 700 dental academics received the invitation. A total of 274 dental academics participated in the study (response rate: 39%).

Questionnaire

An international team²² designed a questionnaire containing 4 sections (the questionnaire is available from the corresponding author on reasonable request). The 1st section referred to background information, and contained 8 questions about the respondent's gender, age, living status, and academic experience,

the number of courses coordinated per semester, the number of students dealt with per semester, the number of daily patient visits, and the respondent's administrative role (having an extra responsibility of managing a department or a certain affair beside the academic role). This section also contained 6 questions on COVID-19-related knowledge with a total of 29 true-or-false statements.²⁶ The 2nd section was about concerns and it contained 18 items; the respondent had to indicate on a 10-point scale how much the particular issue concerned them during the COVID-19 pandemic – from 1 (not worried at all) to 10 (extremely worried). The 3rd section was related to the evaluation of stress and was based on the 15-item Impact of Event Scale (IES).^{27,28} It measured the psychological impact of the COVID-19 pandemic with the 4-point Likert scale response alternatives: 0 – not at all; 1 – rarely; 3 – sometimes; and 5 – often. The sum of points for all the items depicted the stress level of each participant, later categorized as follows: 0–8 – subclinical stress; 9–25 – mild stress; 26–43 – moderate stress; and ≥ 44 – severe stress.²⁸ The 4th section was devoted to changes in behavior and it contained 5 statements the respondent had to refer to, indicating to what degree the particular change occurred in their life during the COVID-19 pandemic – from 1 (extremely low) to 10 (extremely high). The sum of scores for the 5 statements made up the total behavior change score, and the median (*Me*) of this score served as the cut-off between desirable and undesirable behavior changes.

Two English experts translated the questionnaire into Persian, and then back-translated it into English in order to make sure of an accurate translation. The face and content validity of the stress section had been ensured previously.²⁷ However, 5 dental academics who were not involved in the study rated the necessity, relevancy, clarity, and simplicity of items in sections 3 and 4, and recommended some improvements; as a result, the revised version of the questionnaire was developed. We also ran a pilot study on 10 dental academics who were excluded from the study; the questionnaire was administered twice at a 2-week interval to assess its face and content validity as well as reliability, and to make sure of an acceptable agreement. Cronbach's alpha for sections 2–4 after completing the main study was 0.893, 0.793 and 0.866 respectively, all values showing desirable internal consistency.

We uploaded the survey on Google Forms, which is a platform for creating and sharing online surveys. On the 1st page, we explained the study's objectives, and assured the respondents of the voluntary nature of the study participation and data confidentiality. The respondents provided their consent on the 1st page of the questionnaire. The participants could select only 1 option per item and answering all the questions was necessary for submission; therefore, there was no missing data.

Statistical analysis

The IBM SPSS Statistics Windows software, v. 21.0 (IBM Corp., Armonk, USA), was used for data analysis. Absolute and relative frequencies (n (%)), along with means and standard deviations ($M \pm SD$) for normally distributed data, and medians and quartiles (Me (Q_1 – Q_3)) as well as ranges for not normally distributed data served as descriptive statistics. The percentage of correct responses with regard to the overall 29 COVID-19-related knowledge statements was considered as the knowledge score for each respondent.

The principal component analysis (PCA) was conducted for the section regarding concerns after checking the prerequisites; the number of participants was more than 100,²⁹ the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy was 0.843 (above the desired value of 0.6) and Bartlett's test of sphericity was statistically significant ($p < 0.001$).³⁰ The number of extracted components was primarily based on eigenvalues > 1 , but then we also fixed the analysis with 1 factor more or less to find out whether a more reasonable division of items is attainable. We used the rotated component matrix, applying the varimax rotation method with Kaiser normalization to display the result of PCA.

The backward stepwise multilevel linear regression analysis was used to discover the association of background data, concern components and knowledge with the stress level. In addition, we used the backward stepwise multilevel logistic regression analysis to find the association of background data, concern components, knowledge, and the stress score with the behavior change level. The significance level was set at 5%.

Results

Background information, stress and behavior changes

The majority of the respondents were female (66.4%) and the mean age was 39.44 ± 8.89 years. Besides, the mean COVID-19-related knowledge score was 74.30 ± 9.63 . The fully detailed background information about the participants is presented in Table 1.

Table 1 also demonstrates the mean COVID-19-related stress scores, the percentages of the stress level with regard to the IES categories and the percentages of the undesirable/desirable behavior change level in dental academics according to background variables. The percentage of participants with moderate to severe stress was 50.4%. Since the behavior change data was not normally distributed, the *Me*, interquartile range (*IQR*) and range values for this section are presented in Table 2. The least behavior change due to the COVID-19 pandemic was observed in 'changing life habits' ($Me = 8$; $IQR = 3$), which was the only item with $Me < 10$. The total behavior change *Me* was 46.5 ($IQR = 8$).

Table 1. Iranian dental academics' ($N = 274$) COVID-19-related stress and behavior change levels according to background variables

Variable	n (%)	Stress level [†] $M \pm SD$	Stress level [‡]				Behavior change level		
			subclinical %	mild %	moderate %	severe %	undesirable %	desirable %	
Gender	male	92 (33.6)	23.39 \pm 11.47	7.6	48.9	38.0	5.4	60.9	39.1
	female	182 (66.4)	27.10 \pm 12.38	5.5	40.7	44.0	9.9	44.5	55.5
Age [years]	25–35	107 (39.1)	25.60 \pm 10.12	0.9	46.7	49.5	2.8	47.7	52.3
	36–45	106 (38.7)	26.61 \pm 12.84	9.4	39.6	40.6	10.4	50.0	50.0
	≥ 46	61 (22.3)	24.98 \pm 14.29	9.8	44.3	31.1	14.8	54.1	45.9
Living status	parents	42 (15.3)	27.90 \pm 12.44	7.1	31.0	57.1	4.8	42.9	57.1
	partner/spouse	198 (72.3)	25.27 \pm 11.76	6.1	46.0	39.9	8.1	50.5	49.5
	other	34 (12.4)	26.74 \pm 14.21	5.9	44.1	35.3	14.7	55.9	44.1
Academic experience [years]	<5	113 (41.2)	25.18 \pm 10.49	2.7	45.1	49.6	2.7	46.9	53.1
	5–10	91 (33.2)	26.64 \pm 12.53	7.7	40.7	40.7	11.0	56.0	44.0
	11–20	41 (15.0)	26.00 \pm 14.32	9.8	48.8	26.8	14.6	48.8	51.2
	≥ 21	29 (10.6)	25.83 \pm 14.38	10.3	37.9	37.9	13.8	44.8	55.2
Number of courses coordinated per semester	0–1	22 (8.0)	26.41 \pm 11.79	4.5	36.4	54.5	4.5	50.0	50.0
	2	51 (18.6)	23.98 \pm 12.25	7.8	54.9	27.5	9.8	60.8	39.2
	3	55 (20.1)	24.76 \pm 11.94	9.1	40.0	41.8	9.1	54.5	45.5
	4	47 (17.2)	26.96 \pm 10.79	4.3	38.3	53.2	4.3	48.9	51.1
	≥ 5	99 (36.1)	26.78 \pm 13.05	5.1	43.4	41.4	10.1	42.4	57.6
Number of students dealt with per semester	0–49	57 (20.8)	26.81 \pm 12.32	1.8	49.1	35.1	14.0	54.4	45.6
	50–100	122 (44.5)	25.86 \pm 11.60	6.6	40.2	45.9	7.4	52.5	47.5
	101–200	59 (21.5)	24.86 \pm 13.16	10.2	47.5	35.6	6.8	44.1	55.9
	≥ 201	36 (13.1)	25.94 \pm 12.65	5.6	38.9	50.0	5.6	44.4	55.6
Number of daily patient visits	0	56 (20.4)	23.95 \pm 13.28	12.5	42.9	33.9	10.7	44.6	55.4
	1–10	142 (51.8)	26.50 \pm 12.40	4.9	42.3	45.1	7.7	59.9	40.1
	11–20	48 (17.5)	25.75 \pm 10.66	4.2	45.8	43.8	6.3	35.4	64.6
	≥ 21	28 (10.2)	26.57 \pm 11.47	3.6	46.4	39.3	10.7	35.7	64.3
Administrative role	yes	124 (45.3)	24.97 \pm 12.55	9.7	46.0	35.5	8.9	46.0	54.0
	no	150 (54.7)	26.59 \pm 11.87	3.3	41.3	47.3	8.0	53.3	46.7
Total		274 (100.0)	25.85 (12.19)	6.2	43.4	42.0	8.4	50.0	50.0

COVID-19 – coronavirus disease 2019; M – mean; SD – standard deviation; [†] based on the Impact of Event Scale (IES); [‡] based on the IES categories.

Table 2. Behavior changes in Iranian dental academics ($N = 274$) after the onset of the COVID-19 pandemic

Behavior change [†]	Me (Q_1 – Q_3)	Range
Changing life habits	8.00 (7.00–10.00)	1.00–10.00
More frequent handwashing	10.00 (8.00–10.00)	1.00–10.00
Avoiding crowded places	10.00 (9.00–10.00)	1.00–10.00
Cancelling social events	10.00 (9.00–10.00)	1.00–10.00
Cancelling travel plans	10.00 (10.00–10.00)	1.00–10.00
Total behavior change [‡]	46.50 (42.00–50.00)	5.00–50.00

Me – median; Q_1 – 1st quartile; Q_3 – 3rd quartile; [†] out of 10 for each item; [‡] out of 50.

Dental academics' concerns regarding COVID-19

Table 3 presents COVID-19-related concern items and the rotated component matrix of PCA with loadings between

0.627 and 0.845 in 3 components. The greatest and the least 'fear of infection' scores for the participants were respectively 'loved ones getting infected with COVID-19 because of me' (8.96 \pm 1.87) and 'catching the COVID-19 infection from a student' (6.42 \pm 2.74), while the mean total score for this component was 53.51 \pm 11.89 out of 70. The greatest and the least 'concerns over professional responsibilities' scores for the participants were respectively 'supporting students psychologically in the difficult times of the COVID-19 outbreak' (6.62 \pm 2.45) and 'finishing open courses satisfactorily during the COVID-19 outbreak' (6.36 \pm 2.84), while the mean total score for this component was 32.70 \pm 10.78 out of 50. The greatest and the least 'concerns over restrictions' scores for the participants were respectively 'economic impact caused by the COVID-19-related lockdown' (7.99 \pm 2.27) and 'missing scientific events important to my career because of the COVID-19 outbreak' (5.31 \pm 2.91), while the mean total score for this component was 37.37 \pm 12.38 out of 60.

Table 3. Principal component analysis (PCA) for the COVID-19-related concerns of Iranian dental academics ($N = 274$)

Concerns	Score [†] $M \pm SD$	Components		
		fear of infection	concerns over professional responsibilities	concerns over restrictions
Catching the COVID-19 infection from a colleague at work	6.56 ± 2.60	0.730	–	–
Catching the COVID-19 infection from a patient during treatment	8.49 ± 2.02	0.726	–	–
Catching the COVID-19 infection from a student	6.42 ± 2.74	0.715	–	–
Catching the COVID-19 infection from a source not related to work	6.73 ± 2.53	0.678	–	–
Loved ones getting infected with COVID-19 because of me	8.96 ± 1.87	0.689	–	–
Loved ones getting infected with COVID-19 because of another source	8.44 ± 1.95	0.678	–	–
Patients getting infected with COVID-19	7.92 ± 2.32	0.682	–	–
Finishing open courses satisfactorily during the COVID-19 outbreak	6.36 ± 2.84	–	0.820	–
Teaching students the required material during the COVID-19 outbreak	6.56 ± 2.65	–	0.845	–
Supporting students psychologically in the difficult times of the COVID-19 outbreak	6.62 ± 2.45	–	0.641	–
Managing the extra load of online lecturing during the COVID-19 outbreak	6.60 ± 2.68	–	0.760	–
Finishing the required reports, assignments and duties before deadlines during the COVID-19 outbreak	6.56 ± 2.59	–	0.800	–
Restricted mobility from one place to another in my country because of the COVID-19 outbreak	6.07 ± 3.10	–	–	0.715
Restricted mobility from and to my country because of the COVID-19 outbreak	5.52 ± 3.18	–	–	0.736
COVID-19-related restrictions affecting my sports and social activities	6.63 ± 2.61	–	–	0.646
Missing scientific events important to my career because of the COVID-19 outbreak	5.31 ± 2.91	–	–	0.699
Short supplies for personal and/or household use because of the COVID-19 outbreak	5.85 ± 2.88	–	–	0.714
Economic impact caused by the COVID-19-related lockdown	7.99 ± 2.27	–	–	0.627

Rotated component matrix with the use of the varimax rotation method with Kaiser normalization. [†] out of 10 for each item.

Factors associated with stress and behavior changes

Table 4 and Table 5 demonstrate the factors associated with the COVID-19-related stress and behavior change levels, respectively. As there were interactions between gender and other associated factors in both regression models, we considered gender as an effect modifier, and analyzed male and

female dental academics separately after ensuring an adequate statistical power with the use of the Power Analysis and Sample Size (PASS) software, v. 15 (NCSS, Kaysville, USA). As for the gender itself, women demonstrated more stress (mean difference (MD): 3.708; 95% confidence interval (CI): 0.665–6.750; $p = 0.017$, according to the independent samples t test) and more desirable behavior (odds ratio (OR): 1.940; 95% CI : 1.164–3.232; $p = 0.011$, according to the χ^2 test).

Table 4. Factors associated with Iranian dental academics' COVID-19-related stress level (based on the Impact of Event Scale (IES)) according to gender

Variable [#]		Coefficient	95% CI	p -value	
Men $n = 92$	partner/spouse	ref.	–	–	
	living status	parents	12.215	4.125–20.306	0.004*
		other	2.858	–3.466–9.181	0.372
	fear of infection		0.193	0.006–0.381	0.044*
	concerns over restrictions		0.238	0.047–0.429	0.015*
Women $n = 182$	academic experience [years]	<5	ref.	–	
		5–10	4.188	0.356–8.021	0.032*
		≥11	7.221	2.464–11.978	0.003*
	administrative role	yes	ref.	–	
		no	4.573	1.083–8.063	0.011*
	fear of infection		0.252	0.084–0.420	0.004*
concerns over restrictions		0.228	0.074–0.382	0.004*	

Backward stepwise multilevel linear regression analysis. Power analysis: $p0^2 = 0$; the saturated model's R^2 values of 0.323 (men) and 0.246 (women); a power of 99% (men) and 100% (women).

CI – confidence interval; ref. – reference category which other categories of a particular variable were compared with in the regression model; [#] only the significantly associated variables which remained in the final regression model for each gender are presented in the table; * statistically significant.

Table 5. Factors associated with Iranian dental academics' COVID-19-related behavior change level according to gender

Variable [#]			OR	95% CI	p-value
Men n = 92	administrative role	no	ref.	–	–
		yes	3.079	1.156–8.200	0.024*
	fear of infection		1.096	1.045–1.150	<0.001*
Women n = 182	number of daily patient visits	0	2.010	0.915–4.414	0.082
		1–10	ref.	–	–
		≥11	3.638	1.633–8.107	0.002*
	fear of infection		1.063	1.030–1.097	<0.001*
	knowledge		1.051	1.008–1.095	0.019*

Backward stepwise multilevel logistic regression analysis (category 0 – undesirable level of behavior change; category 1 – desirable level of behavior change). Power analysis: $p0^2 = 0$; the saturated model's pseudo R^2 values of 0.323 (men) and 0.174 (women); a power of 99% (men) and 99% (women).

OR – odds ratio; ref. – reference category which other categories of a particular variable were compared with in the regression model; [#] only the significantly associated variables which remained in the final regression model for each gender are presented in the table; * statistically significant.

Greater fear of infection and concerns over restrictions were associated with more stress in both men ($p = 0.044$ and $p = 0.015$, respectively) and women ($p = 0.004$ and $p = 0.004$, respectively). Furthermore, men living with their parents demonstrated more stress due to the COVID-19 pandemic than those living with their partner/spouse ($p = 0.004$). Regarding women, those with less than 5 years of academic experience demonstrated less stress as compared to those with 5–10 years ($p = 0.032$) and 11 or more years ($p = 0.003$) of academic experience, much like administrators, who demonstrated less stress than those without any administrative role ($p = 0.011$) (Table 4).

In both men and women, a greater fear of infection was associated with desirable behavior changes due to the COVID-19 pandemic ($p < 0.001$ in both cases). In addition, desirable behavior changes were more evident in men with administrative roles ($p = 0.024$) and more knowledgeable women ($p = 0.019$), while undesirable behavior changes were more apparent in female dental academics visiting 1–10 patients daily as compared to those visiting 11 or more patients per day ($p = 0.002$) (Table 5).

Discussion

The repercussions of the rapid spread of COVID-19, ranging from isolation and quarantine to disease contraction and death, have resulted in undeniable psychological fear and stress. Healthcare workers, particularly dentists, who continuously treat patients, are at higher risk of contracting infectious diseases, which increases the possibility of distress even further.^{5,15} Dental academics are additionally affected by the closure of dental schools.¹⁶ The World Health Organization (WHO) has recommended lifestyle changes, such as crowd avoidance, more frequent handwashing, and the cancellation of travel plans and events to control the infection.³¹ It is important that dental academics adhere to these recommendations, as they practice in a high-risk environment, and also serve

as profession and community role models. However, psychological distress can cause irrational behavior, along with physical and mental health problems.³² We aimed to provide insight into the early impact of the COVID-19 pandemic on dental academics' psychological state and behavior, and distributed an online questionnaire focusing on concerns, stress and behavior changes during the COVID-19 pandemic for this purpose.

We divided dental academics' COVID-19-related concerns into 3 different components with the use of PCA: the fear of infection; concerns over professional responsibilities; and concerns over restrictions. The greatest concern expressed by the participants referred to the fear of becoming infected by their patients and spreading the virus to their loved ones, which is in line with other studies.^{24,33–35} Similar to our study, Shacham et al. reported that dentists were more concerned about themselves as compared to their patients regarding disease contraction.²⁴ Ahmed et al. suggested that meticulous adherence to the recommendations of regulatory authorities could reduce dentists' fear of infection.³³ The least concerns of our study participants were the ones about the restrictions regarding mobility and events; however, the respondents were concerned about the economic impact of the lockdown, much like Mishra et al.'s study participants.³⁵ Dentists have been one of the most economically affected categories of health professionals during the pandemic.³⁶ On the other hand, similar to our study, Martina et al. reported that concerns over the economic loss and quarantine were of less importance to dentists than the fear of infecting family members.³⁷

In the regression models, we analyzed men and women separately, as gender interacted with some other important factors; however, we did not compare concern between genders. Interestingly, Gasparro et al. reported that higher levels of COVID-19-related fear and concern were evident in female dentists.³⁸

More than 90% of our participants were experiencing psychological distress, similar to dentists in a study by Ranka and Ranka,³⁹ which highlights a vital need for

supportive efforts. Furthermore, 8.4% of our study respondents demonstrated severe stress. Similar studies reported the percentage of severe stress, anxiety or depression to be 9.9% among dental academics from 28 countries,²² 8.7%, 6.4% and 5.4% in Italian dentists (3 studies),^{34,37,40} 22% and 17.9% in Indian dentists (2 studies),^{35,41} 5.2–8% in German dentists,⁴² and 2% in Indian medicine, dentistry, nursing, and physical therapy academics.⁴³ Other studies also reported 11.5–25.7% of dental professionals experiencing elevated psychological impact.^{24,44} Differences in percentages are probably due to different measurement scales and country conditions. Although these proportions are small, extensive psychological attention is of great importance to these groups. Interestingly, dentistry and physical therapy academics demonstrated higher levels of stress as compared to medicine and nursing academics.⁴³

Women in the present study demonstrated a worse COVID-19-related psychological outcome, similar to other studies among dentists.^{38,41,42} Previous studies claimed that women experienced higher levels of depression and anxiety as compared to men during the COVID-19 pandemic in the general population,⁴⁵ which may have originated from the overall gender differences already reported for anxiety and depressive symptoms.⁴⁶ However, some studies reported no difference between male and female academics⁴³ and dental professionals^{24,47} regarding COVID-19-related distress, and attributed their results to the governmental support for women. Therefore, the need for additional support systems for female dentists is undeniable in Iran.

In the present study, stress among women was less evident in administrators, probably due to being more in charge, and also less experienced ones, possibly due to the lesser fear of death. The underlying medical conditions, which are more evident in older dentists, have also been associated with more distress during the COVID-19 pandemic.^{24,42} In contrast, younger and less experienced academics and dental professionals from several other countries demonstrated higher levels of psychological impact^{35,38,42–44} due to the added responsibility of balancing the family life and finances, less developed coping skills, and more mobility and time spent on social media.⁴⁸ Those studies mostly did not control for other factors, whereas we used a regression model to control for other variables. Greater subjective overload and financial concerns, which are more evident in younger dentists, have also been associated with a greater psychological impact.^{24,41,42} Conversely, Shacham et al. reported that there were no association between age and COVID-19-related distress.²⁴ These contradictions probably originate from different scales for the psychological impact measurement and different conditions in different countries.

In the present study, men who lived with their parents were significantly more stressed as compared to those living with their partner/spouse. A possible solution is

to leave parents and live on their own; still, a longitudinal study should back up this solution with evidence. Our result is in line with the findings of Shacham et al., who reported that dental professionals who were in a committed relationship demonstrated less distress.²⁴ This is probably due to a greater risk of COVID-19 in older parents, against the great emotional support of the partner and their buffering effect.⁴⁹ In contrast, no differences in terms of psychological outcomes existed between various marital statuses in Mekhemar et al.'s study.⁴²

Concerns over infection and restrictions were positively associated with stress in the participants of the present study. Similarly, other studies introduced the fear of contracting and spreading COVID-19 to close persons^{22,24,34,38,41} as well as restrictions during the pandemic²² as stressors and depressors to dental professionals. Ammar et al. also highlighted the association of worries about the professional responsibilities related to teaching and research with stress during the COVID-19 pandemic,²² as the lack of fulfillment at work can negatively affect mental health.⁵⁰ On the other hand, such associations did not exist in our study. Another interesting finding of the present study is that the association between the fear of infection and stress was much stronger in women than men.

Dental academics in the present study demonstrated overall acceptable behavior change due to the COVID-19 pandemic. The participants reported that they definitely washed their hands more frequently, avoided crowded places, and canceled social events and travel plans after the onset of the COVID-19 pandemic; however, they were fairly hesitant in changing their life habits. On the other hand, 12.8% of American dental hygienists attended large public events during the pandemic.⁴⁴ Professionals should not only avoid crowded places and social events themselves, but also encourage the public to do so.

The present study has 2 distinct features with regard to the behavior change section which distinguish it from similar studies. Firstly, it focuses on general protective behaviors, not the preventive measures used in dental practice. Secondly, it measures the amount of change in protective behaviors due to COVID-19 rather than the behaviors during the pandemic themselves, therefore omitting the effect of difference in individuals' protective behaviors before the pandemic.

The behavior change of male dental academics in our study was less desirable as compared to females; however, male administrators' behavior change was more desirable as compared to non-administrators. This may be due to their attitude as a role model for other academics. Furthermore, women visiting more patients daily had changed their behavior more desirably as compared to those whose offices were open, but who visited fewer patients. In addition, the fear of infection had a positive association with desirable behavior change in all responders, contrary to knowledge, which showed such a positive association only in women. This brings the idea

of increasing COVID-19-related knowledge to induce better behaviors; however, this hypothesis must be evaluated in a randomized study. Similarly, negative emotions, such as fear, worry and anxiety, and also higher fatality rates of the country have proved to be positively associated with the COVID-19-preventive behaviors of dentists, such as handwashing and social distancing.²² The promotion of preventive behaviors could help those with undesirable behavior change.

One of the strengths of the present study is its psychometric quality, which shows high internal consistency of the questionnaire, reliable scales and high statistical power due to the large sample size from among Iranian dental academics. Another strength is the robust methodological approach of the study, which contained PCA and effect modification. The present study portrayed the psychological impact of the COVID-19 pandemic at its early stages; therefore, it can be useful in case of similar pandemics in the future. This study may also help policymakers to make better decisions and enhance the resiliency and preventive behaviors of dental academics during the COVID-19 pandemic.

Limitations

The present study also has some inevitable limitations, such as a fairly low response rate due to the online nature of the study. Furthermore, female and young dental academics made up the majority of our respondents, which may be due to more time spent online by them. Therefore, the possibility of self-selection bias exists; however, we used various routes and platforms for sending the invitation to diminish the errors of convenient sampling. Our study's fairly large sample could represent almost all Iranian dental academics. However, one should keep in mind that dental academics and specialists have proved to experience less stress during the pandemic than other dentists do.³⁵ Generalizing the results to all dental academics in the world is not possible, as the amount of fear and distress due to COVID-19 as well as the level of associations differ in each country.⁴⁷ In addition, the cross-sectional nature of the study resulted in overlooking changes in psychological impact through time and the elimination of the cause–effect relationship.

Conclusions

The present study demonstrated the great psychological impact of the COVID-19 pandemic on Iranian dental academics, with moderate to severe stress being apparent in about half of them. However, most of the participants had effectively changed their behavior during the pandemic. The fear of getting infected and spreading the virus to the loved ones was a major concern, which had a positive association with stress and behavior change. Concerns over

mobility and other restrictions was less of an issue for the respondents; however, they were positively associated with stress. Female dental academics were more stressed, but presented more desirable behavior change. This study identified more vulnerable dental academics, who must be the target of psychological and behavioral, supportive and educational programs. We recommend conducting similar studies on dental students, as they are having a hard time studying and socializing. It is also reasonable to conduct prospective or randomized studies to investigate different methods of stress reduction and desirable behavior promotion among HCWs.

Ethics approval and consent to participate

The research was approved by the institutional ethics committee (approval ID: IR.TUMS.DENTISTRY.REC.1399.001) at Tehran University of Medical Sciences, Egypt. Informed written consent was obtained from the participants before filling in the questionnaire.

Data availability

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

Consent for publication

Not applicable.

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Bacterial contamination on the inner surface of the masks used by clinicians in a dental clinic

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Abstract

Background. Wearing masks has become an essential safety measure during the coronavirus 2019 (COVID-19) pandemic. Especially clinicians in dental clinics should focus on infection control. However, there is currently no data regarding the frequency of replacement of the masks used in hospitals. Hence, it is necessary for dental clinic staff, who deal with many patients in an aerosol-producing environment, to have precise mask-wearing guidelines.

Objectives. The aim of this study was to evaluate the contamination on the inner surface of the masks used by clinicians in dental clinics and provide basic data to describe hospital infection control guidelines in greater detail.

Material and methods. For Study I, 12 participants were recruited. The experiment was conducted indoors. After wearing the mask, the temperature and the humidity inside the mask were measured at the set time points (immediately after wearing the mask, and 10 min, 30 min, 1 h, 2 h, and 4 h after wearing the mask). During the experiment, talking was not allowed. For Study II, 23 clinicians from dental clinics were recruited. After wearing the mask, the temperature, the humidity and the number of colony-forming units (CFU) inside the mask were measured at the set time points. Finally, 15 samples were selected for the study based on a survey. Bacterial identification was conducted using the 16S rDNA sequencing.

Results. The temperature and the humidity inside the mask increased significantly relative to room temperature and room humidity, respectively. Bacteria were found in all the masks worn for more than 10 min and the CFU values increased with the mask-wearing time. Bacteria belonging to the genera *Staphylococcus*, *Bacillus* and *Roseomonas* were detected in the used mask.

Conclusions. A mask should not be worn for more than 2 h; however, the frequency of mask replacement can vary, depending on the number of patients encountered and the working environment.

Keywords: bacterial contamination, mask contamination, inner surface of masks

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Introduction

During the coronavirus disease 2019 (COVID-19) pandemic, wearing masks has become an essential safety measure to protect oneself and others.¹ However, patients who visit dental clinics have to take off their masks and keep their mouths open during treatment. In addition, the handpieces and the ultrasonic equipment used in dental clinics produce aerosols that are contaminated with the patient's saliva and blood.² Therefore, dental clinic staff should focus on infection control. With regard to personal protective equipment (PPE) for infection control, it is reported that masks can filter 60–95% of aerosols.² However, the filtering effect depends on the material and shape of the mask; if the fit is incorrect, the unfiltered microorganisms can penetrate through the mask.³ Various microorganisms, such as bacteria,^{4–8} viruses^{9,10} and fungi,⁸ have been detected in masks. The contamination of masks by these microorganisms comes from the surrounding environment⁸ or from individuals.^{4,10}

It is predicted that mask contamination is greater on the inside than on the outside,^{4,5} since in the former case, the mask is in direct contact with the human skin and oral microorganisms may be captured by the mask during conversation. In addition, considering the temperature and humidity factors, essential for bacterial growth, breathing allows the maintenance of relatively warmer temperatures and higher humidity inside the mask than outside it.

If the mask is contaminated, it is replaced with a new one to reduce the risk of infection. However, most people use masks daily. In addition, most clinicians replace gloves on a patient-by-patient basis, but the mask is often used for the entire day.

One study confirmed that pathogenic bacteria accumulated when both surgical and cotton masks were worn for more than 4 h,¹¹ and according to the Australian Dental Association (ADA) infection control guidelines, the filtration capacity of the mask starts to decrease after about 20 min, as moisture fills the inner and outer surfaces of the mask.¹² According to the World Health Organization (WHO) recommendations, medical masks are for a single use only, and once used, they should be discarded immediately.¹³

Hence, it is necessary for dental clinic staff, who deal with many patients in an aerosol-producing environment, to have precise mask-wearing guidelines.

Therefore, the aim of this study was to evaluate the contamination on the inner surface of the masks used by clinicians in dental clinics. This study provides basic data to describe hospital infection control guidelines in greater detail.

Participants and methods

This study was approved by the Ethics Committee of Ulsan College, South Korea (No. 1044363-A-2021-001).

Measurement of changes in temperature and humidity (Study I)

After thoroughly explaining the purpose of the study and its procedures, 12 participants (mean age: 27.2 ± 2.9 years, no medical or dental history of a general disease, free of medication) were recruited having provided written informed consent (Table 1). The experiment was conducted in June 2021, indoors on clear days, except cloudy or rainy days. The windows and the doors were closed, and the experiment started at 9 a.m. The participants were given the same type

Table 1. General characteristics of the Study I participants ($n = 12$)

Variables		$M \pm SD$ or n (%)	
Age [years]		27.2 ± 2.9	
Gender	male	10 (83.3)	
	female	2 (16.7)	
Food consumption habit (favorite food type) [cups per day]	soft	9 (75.0)	
	hard	1 (8.3)	
	sticky	2 (16.7)	
	tough	0 (0.0)	
Beverage consumption habit (intake of water) [cups per day]	1–2	2 (16.7)	
	3–4	7 (58.3)	
	5–7	3 (25.0)	
	≥8	0 (0.0)	
Beverage consumption habit (intake of coffee/tea/drinks) [cups per day]	1–2	12 (100.0)	
	3–4	0 (0.0)	
	5–7	0 (0.0)	
	≥8	0 (0.0)	
Cigarette consumption habit	yes	6 (50.0)	
	no	6 (50.0)	
Oral hygiene habits	frequency of tooth brushing [times per day]	2.9 ± 0.8	
	toothbrush hair type	soft	2 (16.7)
		normal	9 (75.0)
		hard	1 (8.3)
	frequency of toothbrush replacement	every 2–3 weeks	2 (16.7)
		every 2–3 months	10 (83.3)
		every 6 months or less often	0 (0.0)
	daily use of dental floss	yes	2 (16.7)
no		10 (83.3)	

M – mean; SD – standard deviation.

of mask (Korea Filter Anti-Droplet (KF-AD) mask; Hwajin Industry, Yeongcheon, South Korea). The participants were helped to fit the masks to their facial shapes. After wearing the mask, the temperature and the humidity inside the mask were measured with a thermometer (Thermo-Hygrometer Pocket/Digital; Daihan Scientific, Seoul, South Korea) and recorded at the set time points (immediately after wearing the mask, and 10 min, 30 min, 1 h, 2 h, and 4 h after wearing the mask). During the experiment, the participants sat on chairs without talking and stayed comfortable, spending time freely, using books or laptops. The researcher continued to monitor the participants, and if they sneezed, coughed or talked, the experiment was stopped and repeated.

Measurement of the number of bacteria (Study II)

After thoroughly explaining the purpose of the study and its procedures, 23 clinicians working at a dental hospital in Ulsan, South Korea (no medical or dental history of a general disease, free of medication) were recruited having provided written informed consent (Table 2). The experiment was conducted in June 2021, indoors on clear days, except cloudy or rainy days. The windows and the doors were closed, and the experiment started at 9 a.m. The clinicians were given the same type of mask (KF-AD mask). Before distributing the masks, a square shape (1 cm × 1 cm) was drawn in the center of each mask and the mask was ultraviolet (UV)-sterilized for more than 40 min. After sterilization, the masks were sealed in sterile zipper bags and distributed at the dental clinic. To prevent cross-contamination by hands, the clinicians washed their hands and wore exam gloves. The researcher first observed the participants to check for the presence of cross-contamination and immediately replaced the contaminated masks with new ones. The sterilized masks were worn using only the ear loops to avoid touching the inner surface of the mask. The clinicians were helped to fit the masks to their facial shapes. After wearing the mask, the temperature and the humidity inside the mask were measured and recorded at the set time points (immediately after wearing the mask, and 10 min, 30 min, 1 h, 2 h, and 4 h after wearing the mask). During the experiment, the clinicians worked while talking. When the experiment was completed, the masks were collected and replaced with new ones. Each collected mask was placed in a sterile zipper bag and sent to the laboratory.

Final sample selection

The final sample size ($n = 15$; mean age: 23.3 ± 0.7 years) to be considered for the study was

Table 2. General characteristics of the Study II participants (clinicians) ($n = 15$)

Variables		$M \pm SD$ or n (%)	
Age [years]		23.3 ± 0.7	
Gender	male	3 (20.0)	
	female	12 (80.0)	
Workplace	clinic A	3 (20.0)	
	clinic D	2 (13.3)	
	clinic S	2 (13.3)	
	clinic SP	8 (53.3)	
Food consumption habit (favorite food type) [cups per day]	soft	12 (80.0)	
	hard	2 (13.3)	
	sticky	0 (0.0)	
	tough	1 (6.7)	
Beverage consumption habit (intake of water) [cups per day]	1–2	2 (13.3)	
	3–4	7 (46.7)	
	5–7	4 (26.7)	
	≥8	2 (13.3)	
Beverage consumption habit (intake of coffee/tea/drinks) [cups per day]	1–2	14 (93.3)	
	3–4	0 (0.0)	
	5–7	1 (6.7)	
	≥8	0 (0.0)	
Cigarette consumption habit	yes	0 (0.0)	
	no	15 (100.0)	
Oral hygiene habits	frequency of tooth brushing [times per day]	3.0 ± 0.7	
	toothbrush hair type	soft	8 (53.3)
		normal	7 (46.7)
		hard	0 (0.0)
	frequency of toothbrush replacement	every 2–3 weeks	3 (20.0)
		every 2–3 months	11 (73.3)
		every 6 months or less often	1 (6.7)
	daily use of dental floss	yes	8 (53.3)
		no	7 (46.7)

Names of dental clinics are provided as initials (A, D, S, and SP).

determined based on the survey of the clinicians participating in the experiment (Fig. 1). The following cases were excluded: having make-up before wearing the mask ($n = 4$); chewing gum during the experiment ($n = 1$); and coughing or sneezing during the experiment ($n = 3$).

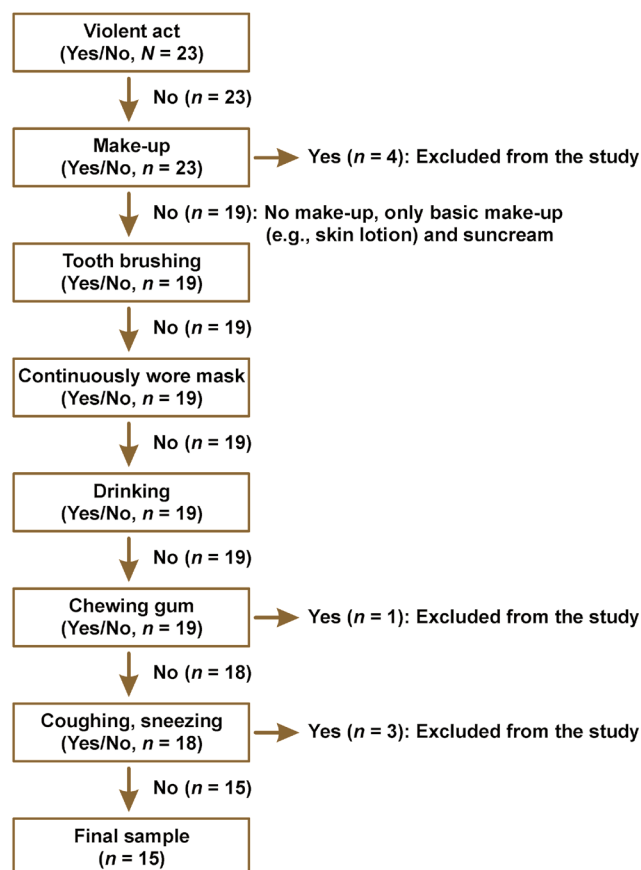


Fig. 1. Flow chart of the sampling process

Y – yes; N – no.

Measurement of general characteristics

The food, beverage and cigarette consumption habits as well as the oral hygiene habits of the participants were surveyed in Study I and Study II (Tables 3,4).

Dental history taking

Both in Study I and Study II, all participants underwent the intraoral examination. Sterile dental mirrors, explorers and tweezers were used at a chair unit. In order to prevent infection, fresh gloves and masks were worn for each examined participant. For the sake of data consistency, the examination was conducted by a single investigator.

For saliva sample collection, the participants were asked to brush their teeth; they were then asked to sit straight in a comfortable chair and their saliva was collected into 50-milliliter conical tubes for a duration of 5 min. After that, the initial saliva pH was measured using a pH meter (PH-200; HM Digital, Redondo Beach, USA). The stimulated saliva secretion rate and the saliva buffering capacity were measured using the saliva-check buffer kit (GC, Tokyo, Japan). Thereafter, the participants were given a chewing gum; after chewing for 30 s, the saliva secreted for a duration of 5 min was collected into a measuring cup to measure the amount of saliva. The collected saliva

Table 3. Oral condition of the Study I participants (n = 12)

Variables			M ±SD or n (%)
Oral health status	gingival bleeding while brushing or flossing	yes	0 (0.0)
		no	12 (100.0)
	bleeding on probing		none
	abnormal condition revealed during the intraoral examination		none
	oral ulcer		none
	gingival swelling or recession		none
	progressive dental caries		none
	preventive dental treatment	yes (sealants)	0 (0.0)
		no	12 (100.0)
	conservative dental treatment	yes (resin fillings)	2.8 ±2.2 8 (66.7)
		no (amalgam fillings)	2.8 ±2.2 4 (33.3)
		no	2 (16.7)
prosthodontic dental treatment		yes (gold inlays)	2.7 ±0.6 3 (25.0)
		yes (gold crowns)	1.0 ±0.0 1 (8.3)
		no	8 (66.7)
	initial saliva pH		7.0 ±0.3
Oral environment	stimulated saliva secretion rate [mL/5 min]		6.9 ±1.6
	saliva buffering capacity		10.3 ±0.5

was dropped onto the tester with the use of a pipette and a change in color was identified; based on this, the buffering capacity test was performed. Color change determination was conducted by 3 independent investigators and an average was calculated (Tables 3,4).

Colony-forming unit (CFU) measurement

The square shape drawn on the mask was cut out and the inner layer was separated using a tweezer. The separated pieces were placed in an Eppendorf tube containing 700 µL of the Luria–Bertani (LB) broth, vortexed for 1 min and incubated for 24 h at 37°C. After dilution (10⁻¹–10⁻⁷), 100 µL was placed onto brain heart infusion (BHI) agar plates. After overnight incubation at 37°C, the number of colony-forming units (CFU) was calculated.

Bacterial identification

Bacterial identification was conducted using a mask worn for 2 h. The masks were directly imprinted for 5 s on BHI agar plates. After incubation for 24 h at 37°C, the colonies were picked randomly and the 16S rDNA sequencing was performed. To amplify 16S rDNA, 2 µL of DNA template and 2 µL of the paired primer set

Table 4. Oral condition of the Study II participants (clinicians) ($n = 15$)

Variables			$M \pm SD$ or n (%)
Oral health status	gingival bleeding while brushing or flossing	yes	0 (0.0)
		no	15 (100.0)
	bleeding on probing		none
	abnormal condition revealed during the intraoral examination		none
	oral ulcer		none
	gingival swelling or recession		none
	progressive dental caries		none
	preventive dental treatment	yes (sealants)	1.6 \pm 1.1 7 (46.7)
		no	8 (53.3)
	conservative dental treatment	yes (resin fillings)	3.4 \pm 2.2 11 (73.3)
		yes (amalgam fillings)	9.5 \pm 0.7 2 (13.3)
	prosthodontic dental treatment	no	3 (20.0)
		yes (gold inlays)	2.3 \pm 1.2 6 (40.0)
		yes (gold crowns)	0 (0.0)
Oral environment	no	9 (60.0)	
	initial saliva pH	6.9 \pm 0.3	
	stimulated saliva secretion rate [mL/5 min]	6.7 \pm 3.6	
	saliva buffering capacity	9.9 \pm 1.3	

(forward primer – 27F: AGA GTT TGA TCM TGG CTC AG; reverse primer – 1492R: GGT TAC CTT GTT ACG ACT TC) were mixed with a polymerase chain reaction (PCR) kit (IP-Taq™ PCR Premix, LaboPass™; Cosmo Genetech, Seoul, South Korea). The reaction mixtures were subjected to 30 cycles of denaturation and annealing at 50°C in an automated thermal cycler (SimpliAmp™, Applied Biosystems/Thermo Fisher Scientific, Beverly, USA). After PCR, the PCR products were resolved by electrophoresis on 1% agarose gel containing the ethidium bromide (EtBr) solution. The final PCR products were purified using the LaboPass DNA Purification Kit (Cosmo Genetech). Finally, the PCR products were analyzed with the automated sequencing, using the BigDye™ Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems), the ABI 3730XL sequencing machine (Applied Biosystems) and a sequencing analysis program (Sequencher™, v. 5.2.4; Gene Codes Corporation, Ann Arbor, USA). The newly aligned 16S rDNA sequences were compared with the bacterial genes deposited in the GenBank® database (National Center for Biotechnology Information (NCBI), Bethesda, USA)¹⁴ and the bacterial strains with 99% matches were searched using the Basic Local Alignment Search Tool (BLAST) for nucleotides (NCBI).

Statistical analysis

The SPSS Statistics for Windows, v. 12.0 (SPSS Inc., Chicago, USA), was used for all statistical analyses. The data was evaluated by means of the one-way analysis of variance (ANOVA), followed by the paired t test. The results of all experiments are presented as mean \pm standard deviation ($M \pm SD$). The statistical significance was set at $p < 0.01$ and $p < 0.05$.

Results

According to the surveys for general characteristics, most of the subjects preferred food of a soft type (Study I: 75.0%; Study II: 80.0%). The amount of water and beverages consumed per day varied with the subjects. None of the subjects in Study II smoked. On average, the subjects brushed their teeth approx. 3 times a day. The preferred toothbrush hair type also varied. The used toothbrushes were replaced every 2–3 weeks (Study I: 16.7%; Study II: 20.0%), every 2–3 months (Study I: 83.3%; Study II: 73.3%), or every 6 months or less often (Study II: 6.7%). Of the total number of subjects, 16.7% in Study I and 53.3% in Study II used dental floss on a daily basis (Tables 1,2).

The intraoral examination was conducted to check the oral health status. Specific intraoral clinical findings, such as gingival bleeding, swelling and recession, were not observed in any of the subjects. In Study I, 66.7% had resin fillings, 33.3% had amalgam fillings, 25.0% had gold inlays, and 8.3% had gold crowns. In Study II, 46.7% had sealants, 73.3% had resin fillings, 13.3% had amalgam fillings, and 40.0% had gold inlays. However, no participants had progressive dental caries. The initial saliva pH (Study I: 7.0 \pm 0.3; Study II: 6.9 \pm 0.3), the stimulated saliva secretion rate (Study I: 6.9 \pm 1.6 mL/5 min; Study II: 6.7 \pm 3.6 mL/5 min) and the saliva buffering capacity (Study I: 10.3 \pm 0.5; Study II: 9.9 \pm 1.3) were normal. The saliva secretion and buffering capacity indicated a healthy status (Tables 3,4).

Upon wearing the mask, the temperature inside the mask increased significantly relative to room temperature ($p < 0.01$). Moreover, the temperature increased even more after 10 min of wearing the mask ($p < 0.01$), and then it remained constant. After 10 min, 30 min and 1 h of wearing the mask, there were significant differences between Study I and Study II; the average temperature in the mask was higher in Study I than in Study II ($p < 0.01$). There were no differences in the temperature in the mask between Study I and Study II after 2 h (Fig. 2).

Upon wearing the mask, the humidity inside the mask increased significantly relative to room humidity ($p < 0.01$). In Study I, the increased humidity remained constant up to 4 h. In Study II, the humidity increased even more after 10 min of wearing the mask ($p < 0.05$) and was maintained for 30 min. However, there was a significant difference

between the humidity values at 10 min and 1 h in Study II ($p < 0.01$). Thereafter, the humidity remained constant up to 4 h. There were significant differences in the humidity values between Study I and Study II at 10 min ($p < 0.01$), 1 h ($p < 0.05$) and 4 h ($p < 0.01$) (Fig. 3).

No bacteria were found in the unused mask sample (control), which was sterilized, enclosed in a zipper bag and returned to the laboratory for examination. Bacteria

were found in all the masks worn for more than 10 min and the CFU values increased with the mask-wearing time. The CFU values increased up to 30 min, but there was no major difference at 30 min as compared to 10 min ($p = 0.055$). However, there were significant differences at 1 h, 2 h and 4 h as compared to 10 min ($p < 0.01$). Significant differences were found after 4 h in comparison with control and other measurement points (10 min, 30 min, 1 h, and 2 h) (Fig. 4).

The bacteria identified in the mask used for 2 h are presented in Table 5. The species were classified in the genera *Staphylococcus*, *Bacillus* and *Roseomonas*.

Discussion

In this study, we selected masks based on the data from previous studies. Dental masks are used most frequently in dental clinics, but according to previous studies, aerosol penetration is significantly higher in dental masks as compared to others.³ Therefore, dental masks were excluded from our study. Furthermore, the filtering capacity of surgical masks was weaker than expected, and thus inappropriate for selection^{3,5,10}; therefore, surgical masks were also excluded. In another study, it was reported that an N95 mask was more uncomfortable than a surgical mask; consequently, participants more frequently touched their mask to move it for comfort (N95: 25 times; a surgical mask: 8 times).¹⁵ Such inconvenience makes it difficult to wear the mask properly, thus increasing its cross-contamination by hands.¹⁶ Therefore, KF-AD masks were finally selected and used in the experiments, since they were better than surgical masks in terms of filtration and more comfortable than the conventional N95 masks.

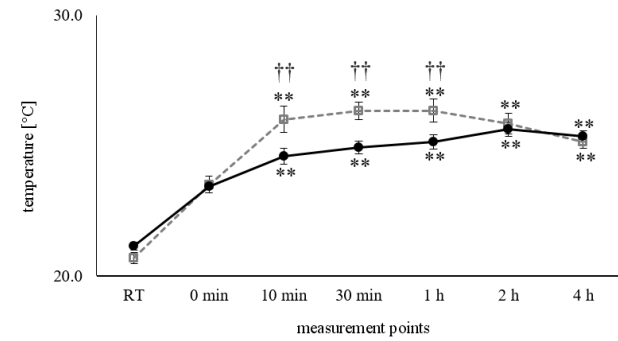


Fig. 2. Temperature changes inside the mask at the set time points Study I – gray dashed line; Study II – black solid line; RT – room temperature; ** $p < 0.01$ (as compared to 0 min); †† $p < 0.01$ (comparison between Study I and Study II).

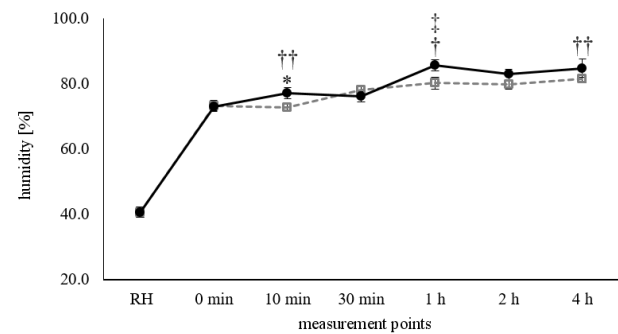


Fig. 3. Humidity changes inside the mask at the set time points Study I – gray dashed line; Study II – black solid line; RH – room humidity; * $p < 0.05$ (as compared to 0 min); † $p < 0.05$ (comparison between Study I and Study II); †† $p < 0.01$ (comparison between Study I and Study II); ‡ $p < 0.01$ (comparison between 10 min and 1 h for Study II).

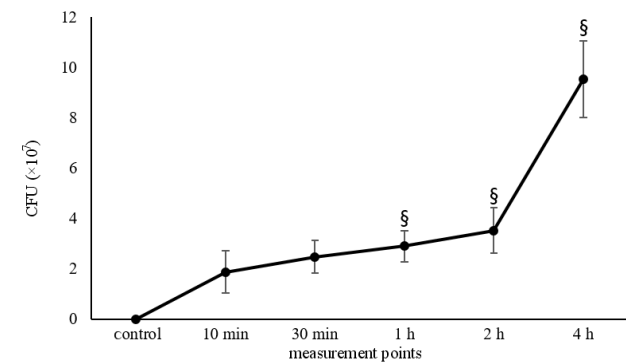


Fig. 4. Changes in the number of colony-forming units (CFU) inside the mask at the set time points § $p < 0.01$ (as compared to 10 min).

Table 5. Bacterial identification in a mask used for 2 h

No.	Bacterial species
1	<i>Staphylococcus</i> sp. strain CLC-M16
2	<i>Staphylococcus epidermidis</i> strain SA144
3	<i>Staphylococcus epidermidis</i> strain L15
4	<i>Staphylococcus</i> sp. U1368-101106-SW107
5	<i>Staphylococcus</i> sp. strain SEP1
6	Bacterium strain BS0580
7	<i>Bacillus</i> sp. L106
8	<i>Bacillus cereus</i> strain BQAR-01d
9	<i>Staphylococcus aureus</i> strain RM_AST_SA012
10	Bacterium NLAE-zl-H450
11	Bacterium NLAE-zl-P761
12	Bacterium strain QLS201804IPB2
13	<i>Roseomonas mucosa</i> strain P37
14	<i>Staphylococcus</i> sp. strain A1
15	Bacterium NLAE-zl-P653

None of the participants consumed liquids during the experiment and continuously wore masks, which might be due to the COVID-19 pandemic. In addition, participants who chewed gum, coughed or sneezed while wearing the mask were excluded from the study to avoid ambiguous results (Fig. 1). One study found that particles in saliva from healthy subjects ranged from 0.09 to 3 μm in size; their concentrations ranged from 100 to 350 particles/L during normal breathing, and from 150 to 2,000 particles/L during talking or coughing.¹⁷ Another study showed the concentrations of particles ranging from 14 to more than 3,000 particles/L and an average particle size of 0.32 μm .¹⁸

It has been reported that upon wearing a mask, the facial and lip temperatures can increase by 5°C and 7.5°C, respectively.¹⁹ In addition, the oral temperature increases after 30 min of wearing a mask.²⁰ Based on these results, we hypothesized that the increased temperature when wearing masks affects bacterial growth and mask contamination. Therefore, we evaluated changes in temperature and bacterial growth with the mask-wearing time.

When comparing the temperature before and after wearing the KF-AD mask, it increased by 2.8°C and 2.3°C in Study I and Study II, respectively. Then, the increased temperature was maintained (Fig. 2). It was confirmed that if the mask was worn correctly, the temperature could significantly increase immediately after wearing the mask. Interestingly, the temperature was considerably higher in Study I than in Study II at 10 min, 30 min and 1 h (Fig. 2), which could be due to the air circulation inside the mask caused by conversation,¹⁵ and warm air may have leaked from the inside of the mask to the outside. There was no further temperature increase when the mask was worn for more than 2 h. If increasing and maintaining the temperature significantly affects bacterial growth, the maximum time of using a mask should be considered as 2 h.

Humidity significantly increased immediately after wearing the mask and was maintained up to 4 h (Fig. 3). This might be due to the moisture trapped from the mouth during breathing and talking. Bacteria can actively grow when a certain amount of humidity is maintained as opposed to dry environments.²¹ Therefore, a shorter mask-wearing time will be better for an individual's health.

The number of CFU on the inside of the mask increased with the mask-wearing time (Fig. 4). Although only a small part was cut off and tested, approx. 1.9×10^7 CFU of bacteria were found at 10 min. It is expected that the bacterial count will be high in the entire mask. After wearing the mask, 2.5×10^7 , 2.9×10^7 , 3.5×10^7 , and 9.5×10^7 CFU of bacteria were found at 30 min, 1 h, 2 h, and 4 h, respectively. These results also indicate that a shorter mask-wearing time should be recommended.

In 230 surgical masks, more bacteria and fungi were detected outside than inside the mask and they were strongly correlated with the air pollution level in the working environment.⁸ A total of 240 masks worn by 130 clinicians during dental treatment for up to 30 min also showed

more bacteria on the outside than on the inside.⁶ However, in the masks from 4 surgeons, the number of bacteria was considerably higher on the inside than on the outside.⁴ This could be due to the environment in the operating room, where internal pollution is efficiently managed and the entry of many people, including medical staff and patients, is strictly prohibited.⁴ After an average of 44 min of aerosol-generating dental treatment, bacteria were detected in the face shield of the dentist carrying out the procedure, with the highest levels of contamination in the inner corner of the eyes and the lateral ala.²² This might be due to the dentist being positioned close to the patient and to the characteristics of dental treatment. The above-mentioned studies suggest that the contamination of PPE can be greatly affected not only by human sources, but also by the surrounding environment. In this study, bacterial contamination outside the mask was not monitored; thus, it was impossible to compare the CFU of bacteria inside and outside the mask. Data on bacterial changes and the working environment of the clinician providing the sample should be obtained in future studies to establish a correlation.

The 16S rDNA sequencing was performed to identify the bacterial species. The detected bacteria were classified in the genera *Staphylococcus*, *Bacillus* and *Roseomonas*.

Staphylococcus epidermidis (*S. epidermidis*) is a gram-positive, facultative anaerobic bacterium; it is part of the normal human flora, typically the skin flora, and less commonly the mucosal flora. *Staphylococcus epidermidis* is not usually pathogenic and infections are generally hospital-acquired.²³

Roseomonas mucosa (*R. mucosa*) is a gram-negative coccobacillus and an opportunistic pathogen that has rarely been reported in human infections. Research on *R. mucosa* has not progressed much; thus, further studies on bacterial infection pathways with the use of masks are necessary.²⁴

Bacillus cereus (*B. cereus*) is a gram-positive, motile, rod-shaped, spore-forming, beta-hemolytic facultative anaerobic bacterium commonly found in soil and food. Some strains are harmful to humans and cause foodborne illnesses, while other strains can be beneficial as probiotics for animals.²⁵

Autoclaving can reduce the effectiveness of mask filters; thus, UV sterilization was selected for the sterilization of masks prior to the experiment. However, no bacteria were found at the initial time point of 0 min, before using the masks, and the masks may have been contaminated with bacteria through an unexpected route after use. This result suggests that there is a possibility of mask contamination by pathogenic bacteria through unexpected sources.

In previous studies, various microorganisms, such as bacteria (including staphylococci^{5–7} and bacilli⁷), viruses⁹ and fungi,⁶ have been detected in masks. The present results confirm that a variety of microorganisms can be found in the contaminated masks. In another study, severe

acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was detected outside the surgical masks worn by the patients infected with the virus.¹⁰ Thus, it is important to note that there is a possibility of the pathogenic microorganisms derived from human sources leaking outside the mask, even if you wear a mask.

Due to different individual health conditions and immune characteristics, the same type of microorganisms can cause diverse problems, such as opportunistic or secondary infections. Thus, it is important to prevent the infections caused by the contaminated masks through increasing the frequency of mask replacement.

Limitations

This study has the following limitations: it was conducted using only one type of mask; anaerobic bacteria were not considered in this experiment; and the respiratory rate and the frequency of conversation at work varied among the participants. Furthermore, the individual skin conditions, such as acne, that could affect bacterial contamination, were not examined.

Conclusions

Based on the results of this study, the following conclusions can be drawn:

- when the mask was worn correctly, the temperature inside the mask significantly increased immediately after wearing the mask; there was no further temperature increase when the mask was worn for more than 2 h. If increasing and maintaining the temperature significantly affects bacterial growth, 2 h should be considered the maximum time of using a mask;
 - humidity significantly increased immediately after wearing the mask and was maintained thereafter. Considering a constant humidity as one of the essential factors for bacterial growth, a shorter mask-wearing time is preferable for an individual's health;
 - the number of CFU on the inside of the mask increased with the mask-wearing time. These results further confirm that a shorter mask-wearing time is beneficial for health;
 - bacteria belonging to the genera *Staphylococcus*, *Bacillus* and *Roseomonas* were detected in the used mask.
- The frequency of mask replacement can vary, depending on the number of patients encountered and the working environment; regular mask replacement will help in infection control.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Ulsan College, South Korea (No. 1044363-A-2021-001). Informed written consent was obtained from the participants prior to the experiments.

Data availability

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

Consent for publication

Not applicable.

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Comparison of bracket bond failure with the aerosol-generating and novel non-aerosol-generating bonding techniques during the SARS-CoV-2 pandemic among orthodontic patients: A retrospective cohort study

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

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Abstract

Background. As per the Centers for Disease Control and Prevention (CDC) guidelines, dentists must avoid the aerosol-generating procedures during the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic.

Objectives. This study aimed to compare the rate of bracket bond failure with the aerosol-generating and non-aerosol generating bonding techniques.

Material and methods. A retrospective cohort study was conducted during the SARS-CoV-2 pandemic on a sample of 44 patients (880 teeth), equally divided into 2 groups: the conventional aerosol-generating bonding technique (ABT); and the novel non-aerosol-generating bonding technique (NABT). The rate of bracket survival and total number of bracket breakages in the 2 groups were assessed by means of the survival regression analysis. The influence of pre-treatment factors, such as the sagittal jaw relationship (ANB), the Frankfort–mandibular plane angle (FMA), overjet, overbite, and upper and lower crowding, on the rate of bracket survival was also evaluated.

Results. There was a statistically significant difference between ABT and NABT in terms of bracket survival. The mean bracket survival with ABT was 176.21 ± 77.89 days and with NABT it was 162.53 ± 83.34 days. The novel technique showed a hazard ratio of bracket survival of 2.99 as compared to the conventional method. There was a statistically significant influence of overjet on the rate of bracket survival ($p = 0.010$). The posterior segment had a higher rate of bracket failure as compared to the anterior segment in NABT, which was statistically significant ($p < 0.001$). However, this difference was statistically non-significant in the case of ABT.

Conclusions. There was an increased rate of bracket failure with NABT as compared to ABT. The rate of bracket breakages in the anterior quadrants was comparable for both bonding techniques.

Keywords: bracket bonding, SARS-CoV-2, orthodontic treatment

Introduction

On March 11, 2020, the World Health Organization (WHO) officially declared coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) a pandemic.¹ The rapid spread of the SARS-CoV-2 viral web is still unpredictable. It is known that COVID-19 is an airborne infection, transmitted by the infected person through droplets and fomites.² SARS-CoV-2 directly influences the nasal and oral epithelium, which results in 95% impairment of taste sensations at the onset of the disease.³ SARS-CoV-2 mainly attacks angiotensin-converting enzyme 2 (ACE2) receptors, which are mostly present in the salivary glands.⁴ The inflammatory response evoked by this infection also leads to severe dryness of the mouth.⁵ Hence, SARS-CoV-2 is not only transmitted by salivary droplets, but saliva is now used for the detection of the virus.⁶

Healthcare professionals, especially dentists, are at higher risk of being directly exposed to the virus due to the inhalation of aerosols and droplets, along with indirect transmission via the contaminated surfaces or by asymptomatic infected patients.⁵ Healthcare facilities are now highly focused on insuring an environment of strict infection control for patients as well as staff members.⁷ Initially, all elective hospital procedures were suspended to minimize the spread of SARS-CoV2.⁸ Routine dental treatment as well as the delivery of orthodontic services were rapidly restricted to only emergency procedures to limit the unwanted risk of exposure.⁹

The WHO promptly presented precise guidelines for emergencies as well as elective dental procedures in order to continue providing patients with healthcare in the infection-controlled environment.¹⁰ As per the Centers for Disease Control and Prevention (CDC) recommendations, dental surgeons as well as orthodontists should limit the aerosol-generating procedures by minimizing the use of high-speed air turbines, ultrasonic scalers or triple syringes.¹¹ If deemed necessary, all the aerosol-generating procedures should be performed while wearing the appropriate personal protective equipment (PPE).¹²

The British Orthodontic Society (BOS), however, classified orthodontic procedures as non-aerosol-generating ones except for the bonding of brackets, the placing of a fixed retainer and the debonding of brackets.¹³ On the contrary, the American Association of Orthodontists (AAO) claims that orthodontists can use slow-speed handpieces and ultrasonic scalers on a completely dry field and in negative pressure operating rooms with high-volume suction.^{10,13} In a study conducted by Golshah et al., high-volume suction reduced the aerosol particles generated during dental procedures by 44%.¹⁴ However, this study examined only the dust particles produced during the aerosol-generating procedures, and not airborne droplets.^{10–13}

It is considered that the laser-assisted procedures, i.e., laser etching, can be regarded as non-aerosol-generating procedures. However, laser etching does not preclude aerosol generating, as it requires water spray as a coolant after etching.¹⁴ Therefore, the rationale of the present study is an urgent need for strict recommendations as to limiting orthodontic procedures to the provision of basic care to patients. Hence, the non-aerosol-generating bracket bonding protocol should be promptly developed. This study aimed to evaluate the rate of bracket breakages with the novel non-aerosol-generating bonding procedure. The secondary objective of this study was to compare the conventional aerosol-generating and novel non-aerosol-generating bonding procedures in terms of bracket breakage. The null hypothesis of this study was that there is no difference in the rate of bracket breakages with the use of the aerosol-generating and the non-aerosol-generating bonding techniques.

Material and methods

A retrospective cohort study was conducted at the Aga Khan University Hospital in Karachi, Pakistan, after obtaining an approval from the institutional Ethics Review Committee (ERC No. 2021-6233-17837). The orthodontic records of bracket breakages among the adult patients who had reported for the initiation of treatment at a tertiary-care hospital were obtained. The sample size was calculated with the OpenEpi (3.0) sample size calculator (<https://www.openepi.com>), based on the findings of Hegarty and Macfarlane, who reported a bond failure percentage of 30% and a risk ratio of 2.6 (1.7, 3.9) for a resin-based composite.¹⁵ Keeping the level of significance (α) at 5% and a power of study ($1-\beta$) of 80%, at least 22 subjects were required in each group with an inflation of 10%. Hence, we included 44 patients and the analysis was done on a total of 880 teeth. Patients above 18 years of age who reported to the orthodontic clinic and initiated fixed orthodontic treatment were included in the study. Those having any syndromes, asymmetries, cross-bites, cleft lip and palate, centric occlusion–centric relation (CO–CR) discrepancy, or with a previous history of orthodontic treatment were excluded from the study.

All the included patients had their brackets bonded for the duration from March 2020 till 2021. The data was collected in April 2021. All patients were given an option of getting the polymerase chain reaction (PCR) COVID-19 testing so that the bonding of brackets by means of the conventional aerosol-generating bonding technique (ABT) (as per the Buonocore protocol¹⁶) could be carried out. This had to be performed under strict infection control conditions to scrutinize symptomatic and asymptomatic patients. We included all those patients who opted for a PCR test as control; however, patients who refused to get tested via a PCR test were bonded by

applying the non-aerosol-generating bonding technique (NABT) as described below and were considered the experimental group.

This novel technique comprised the following steps:

- the tooth surface was cleaned by manual scaling and polished by rubbing with raw pumice on a rubber cup manually instead of using a slow-speed handpiece, an ultrasonic scaler or a triple syringe;
- the enamel surface was etched with 37% phosphoric acid for 15 s, as per the Buonocore protocol¹⁶;
- the etchant was carefully removed from the tooth surface with a cotton roll, preventing damage to the gingiva. Residual acid was removed by cleaning the tooth surface with a damp gauze swab 3 times (each time with a new gauze swab) instead of using high-volume air water pressure from a triple syringe;
- the frosty white surface of enamel was then achieved by high-volume negative pressure suction and tap drying the tooth surface with a gauze swab;
- the primer was then applied on the dried tooth surface and an orthodontic bracket was bonded on the tooth surface with a light-cured composite.

There were 440 teeth in the experimental and 440 teeth in the control group, equally distributed. All patients in the sample were directly bonded with the Roth bracket prescription, a 0.022-inch slot (3M Unitek™; 3M, St. Paul, USA). Leveling and alignment were achieved using round NiTi archwires initially, which were later progressed to 0.018-inch stainless steel (SS) wires during the space closure and molar correction stage. The patients were instructed to maintain oral hygiene, avoid bracket breakages and maintain punctuality with regard to appointments. The teeth were rebonded as soon as the patients reported to the clinic with breakages.

To eliminate any risk of selection bias, all patients with enamel surface defects, missing teeth, early extractions, prematurely debonded or undergoing single-arch treatment, and those noted to be non-compliant were excluded from our study sample.

Other variables, such as the sagittal jaw relationship (ANB), the vertical divergence pattern (SN–GoGn), the lower incisor to the mandibular plane angle (IMPA), and the upper incisor to sella–nasion plane angle (UI–SN) were cephalometrically analyzed; overjet (OJ), overbite (OB), cross-bites, crowding or spacing, and buccal relationships were measured on plaster models with the Mitutoyo multifunctional Vernier caliper (Mitutoyo, Kawasaki, Tokyo).

Statistical analysis

The data was analyzed using the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA), and Stata, v. 12.0 (StataCorp, College Station, USA). The Shapiro–Wilk test showed a normal distribution of the data. Descriptive statistics for continuous variables were

reported as mean and standard deviation ($M \pm SD$). The probability of bracket survival and the factors influencing bracket bond failure in both bonding techniques were evaluated by applying the survival regression analysis. The comparison of the rate of bracket breakages with the use of ABT and NABT was performed by means of the regression analysis.

Results

Demographics

The analysis of descriptive statistics showed an almost equal gender distribution in the ABT and NABT groups. The mean age of the participants was 26.22 ± 9.51 years and it was comparable in both groups (Table 1).

Table 1. Descriptive statistics ($N = 880$ teeth)

Parameter		ABT group ($n = 440$)	NABT group ($n = 440$)
Gender n (%)	female	17 (77.3)	14 (63.4)
	male	5 (22.7)	8 (36.4)
Age [years] $M \pm SD$		26.80 ± 9.74	22.92 ± 4.54
Sagittal pattern (ANB) [°] $M \pm SD$		3.32 ± 2.05	3.86 ± 2.38
Divergence pattern n	hypodivergent	5	3
	normodivergent	11	9
	hyperdivergent	6	10
Arch length discrepancy [mm] $M \pm SD$	upper arch	-2.13 ± 5.58	-1.85 ± 4.53
	lower arch	-1.36 ± 4.24	-2.14 ± 5.13
Overjet [mm] $M \pm SD$		4.04 ± 1.96	5.09 ± 2.84
Overbite [mm] $M \pm SD$		2.72 ± 1.54	3.35 ± 1.67

ABT – aerosol-generating bonding technique; NABT – non-aerosol-generating bonding technique; M – mean; SD – standard deviation.

Bonding technique

Comparing the 2 bonding techniques, we found that the brackets bonded by means of the conventional technique survived for a mean duration of 176.21 ± 77.89 days, with a maximum survival rate of 179.54 days for right upper lateral incisors and first premolars, and for left upper central and lateral incisors and first premolars. However, the brackets bonded by means of NABT survived for a mean duration of 162.53 ± 83.34 days, with a maximum survival of right upper central incisors for 180.27 days (Table 2).

These results were also confirmed with the Kaplan–Meier analysis; in the graph, the blue and the red lines indicate ABT and NABT, respectively (Fig. 1). Establishing ABT as a reference in the regression analysis, we found that there was a statistically significant difference in the rate of bracket breakages between the 2 bonding techniques ($p < 0.001$) (Table 3).

Furthermore, there was a statistically significant difference in the rate of bracket breakages between the anterior and posterior segments ($p < 0.001$). Hence, we assessed the difference between both the segments separately with ABT and NABT. We found that with ABT, the difference in the rate of bracket breakages between the anterior and posterior segments was sta-

Table 2. Bracket survival [days] for both bonding techniques ($N = 880$ teeth)

Parameter	ABT ($n = 440$)	NABT ($n = 440$)
Gender	female	169.27 ± 77.78
	male	156.65 ± 77.91
Divergence pattern	hypodivergent	170.71 ± 52.84
	normodivergent	172.51 ± 85.17
	hyperdivergent	147.39 ± 79.38
Segment	anterior	176.75 ± 76.26
	posterior	163.01 ± 80.66
Arch	maxillary	173.60 ± 84.79
	mandibular	156.90 ± 69.65
Site	right	164.72 ± 79.25
	left	165.78 ± 7.82
RU central incisor	179.37 ± 84.92	180.27 ± 84.12
RU lateral incisor	179.54 ± 84.79	172.77 ± 81.02
RU canine	174.63 ± 83.94	166.47 ± 88.20
RU first premolar	179.54 ± 84.79	173.90 ± 86.34
RU second premolar	147.72 ± 97.13	150.40 ± 96.70
LU central incisor	179.54 ± 84.79	174.18 ± 86.05
LU lateral incisor	179.54 ± 84.79	174.18 ± 86.05
LU canine	166.72 ± 80.59	172.72 ± 88.51
LU first premolar	179.54 ± 84.79	156.77 ± 90.83
LU second premolar	169.90 ± 88.62	147.04 ± 94.65
RL central incisor	159.04 ± 79.69	148.90 ± 74.67
RL lateral incisor	155.22 ± 68.86	155.81 ± 78.87
RL canine	154.13 ± 68.14	167.27 ± 82.48
RL first premolar	159.04 ± 70.69	162.52 ± 88.38
RL second premolar	159.04 ± 70.69	162.19 ± 83.38
LL central incisor	159.04 ± 70.69	167.27 ± 82.48
LL lateral incisor	155.22 ± 68.86	167.27 ± 82.48
LL canine	159.04 ± 70.69	159.33 ± 78.59
LL first premolar	159.04 ± 70.69	165.00 ± 85.38
LL second premolar	150.22 ± 79.90	132.71 ± 64.85

Data presented as $M \pm SD$. RU – right upper; LU – left upper; RL – right lower; LL – left lower.

Table 3. Factors influencing bracket survival ($N = 880$ teeth)

Variables	Hazard ratio	95% CI	p -value	
Age	0.98	0.94, 1.00	0.130	
Overjet	1.08	1.02, 1.20	0.010*	
Overbite	0.89	0.93, 1.24	0.280	
Upper crowding	1.00	0.94, 1.05	0.850	
Lower crowding	0.97	0.94, 1.00	0.130	
Sagittal pattern (ANB)	0.92	0.81, 1.03	0.170	
Group	ABT as reference			
	NABT	2.99	1.60, 5.40	<0.001**
Gender	female as reference			
	male	1.03	0.61, 1.76	0.890
	normodivergent as reference			
Divergence pattern	hypodivergent	0.96	0.55, 1.75	0.960
	hyperdivergent	0.45	0.18, 1.10	0.080
Segment	anterior as reference			
	posterior	3.61	2.07, 6.02	<0.001**
Arch	maxillary as reference			
	mandibular	0.93	0.56, 1.54	0.810
Site	right as reference			
	left	1.04	0.62, 1.74	0.860

Survival simple regression analysis. CI – confidence interval; * $p \leq 0.05$; ** $p \leq 0.001$.

tistically non-significant; however, there was a highly statistically significant difference in the rate of bracket breakages between the segments with NABT ($p < 0.001$) (Table 4).

Cephalometric skeletal variables

The regression analysis revealed that there was a statistically non-significant difference in the rate of bracket breakages with regard to the sagittal jaw relationships in both groups ($p = 0.170$) (Table 3). We also found that in the case of the normodivergent pattern, bracket survival was greater as compared to the hypodivergent and hyperdivergent patterns, as shown in the Kaplan–Meier graph in Fig. 1. However, this difference was statistically non-significant, which indicates that angular values did not influence the duration of bracket survival in any group.

Table 4. Bracket survival for both bonding techniques with regard to the anterior and posterior segments ($N = 880$ teeth)

Group	Variables	Hazard ratio	95% CI	p -value
ABT	anterior as reference			
	segment posterior	2.31	0.82, 6.42	0.110
NABT	anterior as reference			
	segment posterior	4.36	2.24, 8.48	<0.001**

Survival regression analysis. ** $p \leq 0.001$.

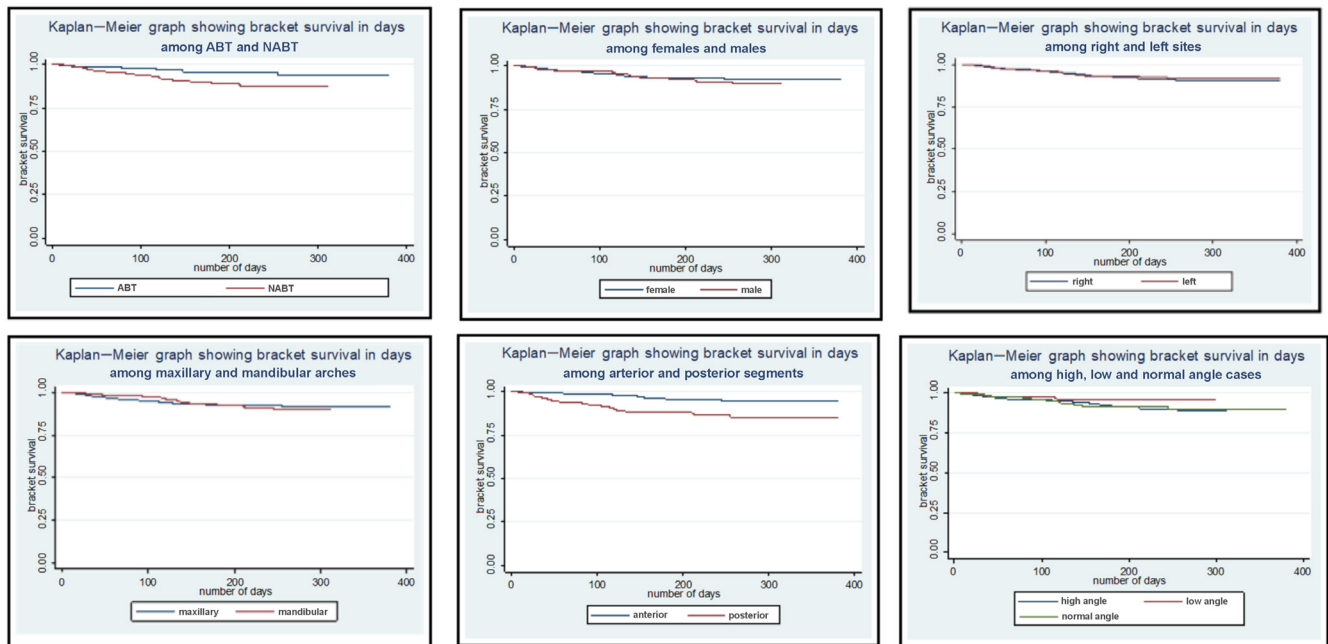


Fig. 1. Graphical comparison of the factors influencing bracket survival

Dentoalveolar variables

Furthermore, we found that with an increase in overjet by 1 mm increased the risk of bracket breakage 1.08 times. Overbite, and upper and lower crowding influenced the rate of bracket breakages insignificantly (Table 3). The rate of bracket breakages was comparable between the right and left sites, with a mean survival of 164.27 ± 83.07 days and 161.74 ± 83.66 days, respectively, with the NABT technique (Table 2). Establishing the maxillary arch as a reference, we found no statistically significant difference in the rate of bracket breakages between the maxillary and mandibular arches, which is also depicted in the Kaplan–Meier graph in Fig. 1; this difference proved to be statistically non-significant in the regression model (Table 5).

Discussion

During the initial months of facing the SARS-CoV-2 pandemic situation, our institution commenced orthodontic bracket bonding by means of NABT. It was required to minimize the risk of spreading the infection through

the salivary splatters produced during the conventional bonding of orthodontic brackets. In the present study, the brackets bonded with ABT survived for a mean duration of 176.21 ± 77.89 days; however, the brackets bonded with NABT survived for an average of 162.53 ± 83.34 days. Apparently, these results lead to the inference that NABT compromised bracket bond strength when compared to the conventional technique. However, we found that there was a statistically non-significant difference in the rate of bracket breakages in the posterior segment with conventional ABT and a highly statistically significant difference with NABT as compared to the anterior segment. This clarifies that in NABT, the posterior segment is the area with a significantly increased rate of bracket breakages. The difference in bracket bond survival in the anterior and posterior segments has also been reported with the use of other techniques related to bracket bonding. While comparing the rate of bracket failure with the use of light-emitting diodes and halogen etching, Krishnaswamy and Sunitha found a difference between the anterior and posterior segments.¹⁷ It has been suggested that the compromised bracket bond strength in the posterior segment is due to an increased risk of salivary contamination and the reduced access to this area.^{17–21} The difference in bond failure rate between the 2 bonding techniques observed in our study could also be due to the lack of experience of dental auxiliary staff in bonding orthodontic brackets with the use of NABT. To insure the quality of treatment, the abovementioned standardized NABT was applied in all the patients from the experimental group. It is because of the effort in standardization that in the anterior region, the results obtained with NABT were comparable to those achieved with the conventional bonding procedure.

Table 5. Factors influencing bracket survival ($N = 880$ teeth)

Variables	Hazard ratio	95% CI	p -value
Overjet	1.10	1.01, 1.20	0.020*
Group	ABT as reference		
	NABT	2.76	1.49, 5.13
Segment	anterior as reference		
	posterior	3.77	2.16, 6.17

Survival multiple regression analysis. ** $p \leq 0.001$.

It is a perceived fact that self-etching bonding and laser etching would minimize the production of aerosol during orthodontic bonding.^{22–26} As reported by Contreras-Bulnes et al., there was a comparable mean duration of bracket survival with the use of self-etching bonding and NABT.²⁶ For laser etching, they obtained similar results as in the case of the conventional bonding technique.²⁶ Self-etching as well as laser-etching bonding techniques do not provide superior results as compared to NABT, as described in our study, and the use of a self-etching system does not preclude the use of a triple syringe, which can lead to developing saliva splatters. Similarly, laser requires water spray as a coolant, which is yet another way of increasing the production of salivary droplets.

It is quite clear from our results that the effectiveness of NABT and ABT for bonding in the anterior segment is comparable. The difference in the rate of bracket bond failure is observable in the posterior segment; the rate may be greater for NABT than for the conventional procedure, but it is still clinically acceptable (Fig. 2) and is of considerable utility when opting for alternative treatment methods during a pandemic situation. According to Skidmore et al., each bracket breakage increases the duration of treatment by 0.6 months.²⁷ Considering this fact, each bracket breakage after every 162 days, would consequently increase the duration of treatment by 0.6 months. This slight delay in treatment can be accepted in contrast to an increased risk of spreading the infection through the production of aerosol, which may lead to an increased rate of mortality in this pandemic situation.

To the best of our knowledge based on a literature search, we were unable to find any study on a bracket bonding protocol entirely with the use of NABT. The non-aerosol-generating bonding technique is an urgent clinical requirement in the present pandemic situation, with the risk of spreading the infection by undiagnosed and asymptomatic patients being increased by 87%.²⁸ The recommendation of this study is that NABT should

be strictly followed in all orthodontic setups during the SARS-CoV-2 pandemic instead of suspending all clinical practices.

Limitations

Apart from the reduced survival of the brackets with NABT, the effect on the quality of enamel, and other pros and cons of this treatment modality need further evaluation with research. Long-term follow-up studies must be conducted to validate the clinical utility of NABT. Since this was a single-center study with a short follow-up period, further studies using this alternative bonding technique need to be conducted in other setups. Another limitation of this study is that the sample was collected in a stressful pandemic situation, which may have increased parafunctional habits (bruxism), leading to an increased risk of bracket breakages. The strength of this study is that 880 teeth were considered for analysis and this large number of teeth validates our study findings.

Conclusions

Through our study on the novel non-aerosol-generating bonding technique (NABT), we found that bracket survival with the use of this technique was 162.53 ± 83.34 days as compared to the 176.21 ± 77.89 days achieved with the conventional bonding technique. The bonding of brackets in the anterior segment was comparable for both bonding techniques; however, there was an increased rate of bracket breakages in the posterior segment with the use of NABT.

Ethics approval and consent to participate

This study was approved by the Ethics Review Committee at the Aga Khan University Hospital in Karachi, Pakistan (ERC No. 2021-6233-17837).

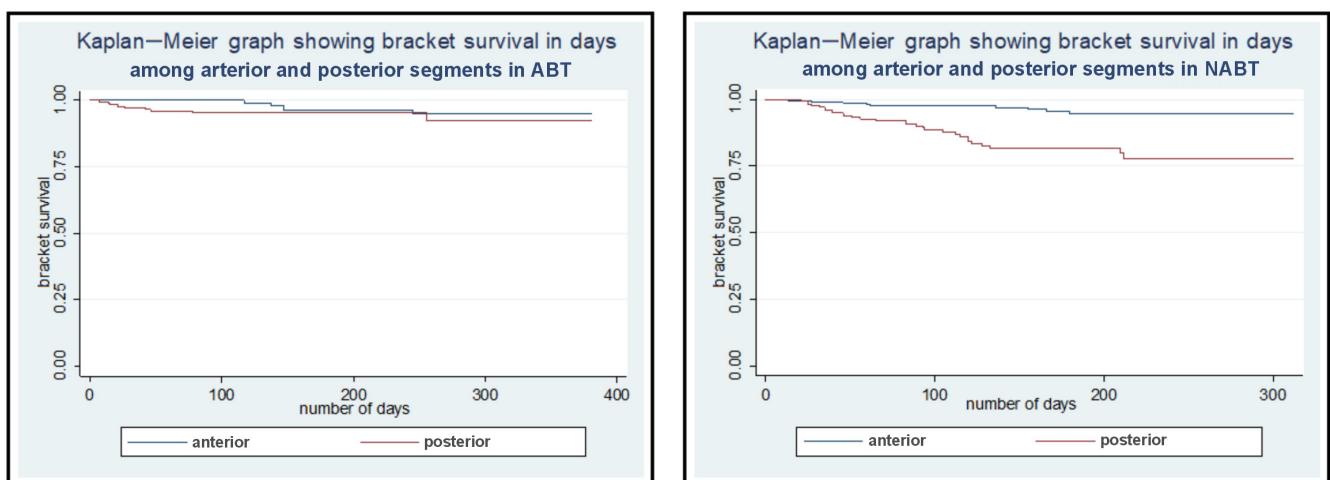


Fig. 2. Graphical comparison of bracket survival in the anterior and posterior segments in both groups





Data availability

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

Consent for publication

Not applicable.

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Infraocclusion level and root resorption of the primary molar in second premolar agenesis: A retrospective cross-sectional study in the Portuguese population

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Conflict of interest

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Abstract

Background. Mandibular second premolar (M2P) agenesis results in the second primary molar (2pm) retention, infraocclusion, a reduced alveolar height and width, the supraeruption of antagonists, or the movement of the adjacent teeth. Infraocclusion affects the survival of the retained 2pm to a greater extent than root resorption.

Objectives. The aim of the study was to evaluate the lifespan of the primary molar as a substitute, with root quality and occlusal adaptation, in cases of M2P agenesis in a low-income population to determine if the attitude of just vigilance could be the best clinical option whenever other clinical problems are absent.

Material and methods. A total of 12,949 orthopantomograms were analyzed. Sixty-one patients (25 males and 36 females aged 7–36 years were divided into group 1 (the first permanent molar in occlusion) and group 2 (the second permanent molar also in occlusion). Vertical positioning to the occlusal plane, root condition and the movement of the adjacent teeth were evaluated.

Results. Despite the study having a cross-sectional design, root resorption, infraocclusion, the distance between the first permanent molar and the first primary molar or the first permanent premolar, and the width of the 2pm were correlated with age. The primary molar root resorption increased with age, which was more pronounced when the second permanent molar was also in occlusion. The mesial movement of the adjacent teeth was absent in all groups. The 2pm was often occluded, but infraocclusion increased with age. Age periods of 11–15 years and 21–25 years are critical for the primary tooth loss.

Conclusions. The second primary molar remains functional in the mandibular arch for up to 25 years. A well-documented no-intervention attitude based on clinical and radiographic data must be weighted in cases without orthodontic issues or with financial constraints.

Keywords: root resorption, infraocclusion, first primary molar, second premolar agenesis, mesial movement

Cite as

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Introduction

Dental agenesis occurs in primary and permanent dentition, usually in the case of third molars, mandibular second premolars, maxillary lateral incisors, and maxillary second premolars,^{1–3} as a sporadic spontaneous de novo mutation⁴ or as familial hypodontia, mainly due to autosomal dominant inheritance,⁵ but also as part of a syndromic condition,⁶ as a phenotypic feature of common conditions, such as Down syndrome or ectodermal dysplasia,^{7,8} isolated or as part of complex syndromes, like labio-palatal cleft^{8,9} or oral-facial-digital syndrome type I.^{7,10}

Other causative factors are environmental factors (radiotherapy, chemotherapy, the disease or infection of the primary tooth, tobacco consumption) or host factors (viral infection during pregnancy, metabolic imbalance).^{11,12}

Different genes are linked with tooth agenesis, including *AXIN2*, *IRF6*, *FGFR1*, *MSX1*, *PAX9*, and *TGFA*.^{13,14} To date, several single-nucleotide polymorphisms (SNPs) and mutations influencing the function of *AXIN2* have been identified and related both to tooth agenesis and colorectal or hepatocellular carcinoma, or prostate, ovary or lung cancer. This supports the hypothesis that missing teeth can be a marker for predisposition to cancer.^{9,13} Agenesis can be diagnosed early in life, allowing the implementation of surveillance programs,^{15,16} as in the case of the demonstrated positive correlation in a three-generation family with an *AXIN2* variant and a history of colorectal cancer, colon polyps and tooth agenesis, probably more as an associated event than as a causative one.¹⁷

The prevalence and severity of dental anomalies are high in humans, and seem jaw- and location-dependent, as most dental anomalies in the maxilla involve the anterior region, and in contrast, the opposite occurs in the mandible, a difference possibly explained by different evolutionary history and ontogeny.¹⁸ Non-syndromic orofacial clefts are frequently associated with tooth abnormalities other than agenesis, such as supernumerary teeth, developmental enamel defects, microdontia, peg-shaped anterior teeth, taurodontism, tooth malposition and/or transposition, tooth rotation, or tooth impaction, but no association with fusion and/or germination was observed.¹⁹

There is evidence of an association between the nutritional status, specifically vitamin D and calcium levels, and severe early childhood caries (S-ECC) in preschool children.²⁰ Still, in severe vitamin D deficiency, there is a high risk of non-syndromic amelogenesis imperfecta and dentinogenesis imperfecta, enamel hypoplasia, hypomineralization/maturation defects, and the abnormal shapes of permanent teeth.²¹ When present, developmental enamel defects are also frequently associated with dental caries in preschool children,²² and clinically occur with discoloration and esthetics problems, tooth sensitivity, wear, and erosion.²³ The main goals of monitoring tooth developmental abnormalities are an early diagnosis, the

improvement of appearance and function, dentition preservation, complication prevention, and the improvement of quality of life.²⁴ The least invasive treatment possible contributes to pulp protection without the further loss of hard tissues, delaying more invasive treatment options as long as possible. Remineralization products alone or combined with CO₂ laser irradiation,²⁵ or CO₂ laser irradiation in different protocols, and resin composites or modified glass ionomer restorations have been suggested to treat the dentinal hypersensitivity associated with dental structure abnormalities.^{26,27}

Mandibular premolar agenesis has been reported as the most common agenesis just after third molars, ranging from 2.4% to 4.3%,^{28,29} with ethnic^{3,30} and gender³¹ variations, revealing its genetic origin,^{4,6} as reported worldwide.^{3,6,30–32} Mandibular second premolar (M2P) agenesis occurs mainly with the retention and infraocclusion of the second primary molar (2pm),³³ the loss of alveolar height and width, antagonist supraeruption, and the movement of the adjacent teeth, with a possible negative influence on the sagittal and vertical dentofacial development, and increased overbites.^{34–36} The loss of space and the retention of first premolars can also occur.²⁸

The 2pm has been described as having one of the longest lifespans.³⁷ Its infraocclusion and root resorption, or the mesial movement of the adjacent teeth seem to slightly increase after 20.³⁸ When present, infraocclusion worsens the prognosis more than root resorption.³⁹ If the 2pm is retained for a long time, its occlusal relationships must be considered, since adequate and well-distributed occlusal forces are crucial for extended survival,⁴⁰ thus correlating longevity with the presence or absence of the second permanent molar.

M2P agenesis should alert to clinically important tooth anomalies, such as an increased risk of agenesis of other permanent teeth, the transposition of incisors, impaction, delayed tooth development, ectopic eruption, retained primary teeth, and different tooth size or shape abnormalities.^{33,41–43}

When treating a skeletal malocclusion, it is difficult to predict the final facial growth, and the challenge becomes even greater in the presence of dental anomalies, which compromise normal function and esthetics.⁴⁴ Articles specifically relating M2P agenesis to skeletal malocclusions are extremely rare and performed in the populations seeking orthodontic treatment. Data revealed inconsistency and dependency on ethnicity. That said, there seems to be some tendency to associate M2P agenesis with Class III^{44,45} or Class II/div 2^{1,46} skeletal malocclusion, and with an hypodivergent growth pattern.

The diagnosis of tooth agenesis and treatment planning involve clinical evaluation and radiographic confirmation.⁴⁷ Radiographic parameters are usually obtained from orthopantomography,^{42,43,48} lateral cephalograms,⁴⁹ bite-wing or periapical radiographs,⁵⁰ and cone-beam computed tomography (CBCT) if the conventional radiography

fails to provide a correct diagnosis, but not as a standard method of diagnosis,⁵¹ considering a more significant radiation risk⁵² and a higher economic cost relative to the conventional radiography.⁵³ In cases with palatal clefts involving complex decisions, like osseous grafts or the need to preserve crucial anatomic structures, CBCT may be required. Combining low mAs (16) and kVp (70) with a small voxel size (180 μ m) enables the association of a low effective dose with high image quality.⁵⁴ More recently, the possibility of using magnetic resonance imaging (MRI) as a feasible tool for orthodontic treatment planning without radiation exposure has been described, by transforming the acquired data into lateral cephalograms, allowing reliable measurements, similar to those applied in orthodontics routine or related disciplines, such as orthognathic surgery, despite the need for specific post-processing software and an experienced user.⁵⁵ Magnetic resonance imaging may also be an alternative diagnostic tool for three-dimensional (3D) cephalometric analysis with an excellent agreement with the reference measurements of CBCT, the accepted gold standard for 3D cephalometric analysis.⁵⁶

The careful examination of orthopantomograms identifies abnormalities in number (hypodontia, oligodontia and hyperdontia), size (microdontia and macrodontia), structure (including amelogenesis imperfecta, dentinogenesis imperfecta and dentin dysplasia), position (transposition, ectopia, displacement, impaction, and inversion) and shape (fusion/gemination, dilaceration and taurodontism), most of them asymptomatic.⁵⁷ Such data is precious in syndromic patients,^{10,58} as these patients need periodical dental and orthodontic supervision to prevent or control the subsequent oral problems.

The early detection of agenesis is crucial for an appropriate and reasonable interceptive treatment plan for a missing M2P.⁴⁹ Mandibular post-rotation and the increased total gonial angle associated with infraocclusion have been described, reinforcing the need for an early diagnosis⁵⁹ and the intervention of a multidisciplinary team.⁶⁰ The 2pm retention, with or without infraocclusion, with the absence of M2P agenesis must be wisely identified, as the treatment plan in the presence of ankylosis is more or less ascertained.⁶¹ Meanwhile, the extraction of the 2pm with a missing M2P may offer benefits, such as avoiding prosthetic replacement, and reducing or eliminating the need for orthodontic appliances once spontaneous space closure occurs, especially if the second permanent molar has not yet erupted.⁶²

In cases with dental crowding, autotransplantation must be considered, as it may have a good prognosis, provided it is carefully planned and timed. In growing individuals, the transplanted tooth enables the alveolar ridge growth and development, and may offer a permanent solution to agenesis,⁶³ mainly because the implant survival in children under the age of 13 is low, with most losses occurring early during the healing phase.⁶⁴ Moreover,

despite decreased passive eruption in patients over 15,⁶⁵ replacement with an implant must be well-weighted, as using implants in growing children is contraindicated,⁶⁶ and to overcome in the future the infraocclusion of the implant-supported crown, a new restoration, orthodontic treatment, distraction osteogenesis, or coronal implant placement is often recommended.⁶⁷ Furthermore, patients with M2P agenesis have narrower and shorter mandibular cross-sections than a control group, with pronounced lingual alveolar plate and submandibular fossa, enhancing the risk of bone perforation during endosseous replacement (tooth autotransplantation or implant installation).⁶⁸ However, this constraint can be minimized with a well-established osseous diagnosis and a 3D additive manufacturing technology.⁶⁹

A fixed prosthesis, either as a permanent partial bridge or a semi-permanent resin-bonded bridge, like an implant, restrains the alveolar process growth, not being a perfect solution. Despite not being focused on M2P agenesis, a study by Cahuana-Bartra et al. revealed that patients with hypodontia showed satisfaction with resin-bonded bridges over a 7-year observation period, with an 88% success.⁷⁰

Regarding treatment options, data from 42 studies published in the years 1980–2015 presented a mean survival of 95.3%, 94.4%, 89.6%, and 60.2% for implants, autotransplants, retained primary teeth, and conventional prostheses, respectively.⁶⁴ Meanwhile, the mean satisfaction rates for the type of treatment, i.e., for implants, conventional prostheses, autotransplants, and orthodontic space closure, 93.4%, 76.6%, 72.0%, and 65.5%, respectively.⁶⁴ Yet, in the last two decades, there seems to be a shift in the therapeutic decision with a tendency to prefer orthodontic space closure to space opening and prosthetic replacement, perhaps reflecting a greater optimism with biomechanical strategies since the implementation of temporary anchorage devices (TADs) to assist in space closure, especially if the agenesis is asymmetrical,⁷¹ as TAD-assisted space closure can be considered a safe treatment option for young patients with M2P agenesis.⁷² Autotransplants and deciduous teeth were reported to have low annual failure rates, and seem appropriate for children and adolescents at a low cost.⁶⁴ The review found a mean observation time of 4.1 years for children, 4.9 years for adolescents (<18 years) and 6.4 years for adults in the included studies.⁶⁴ In cases with the agenesis of multiple teeth, the attachment of an overdenture on the remaining teeth can be considered,⁷³ provided the daily oral hygiene and routine maintenance are feasible.

Concerning M2P agenesis, despite the agenesis being located posteriorly, the patient's self-image can play an essential role in making clinical treatment decisions and the dentist's esthetic judgment.⁷⁴ These patients and their families would probably benefit from an oral health-related quality of life (OHRQoL) questionnaire to accelerate the implementation of treatment. Despite this

kind of agenesis being presumably less esthetically compromising, children with oligodontia were described as having poorer scores as compared even to their parents, with no direct relationship with the number of missing teeth, exhibiting significantly worse social well-being scores for anterior agenesis and better ones whenever there was a retained primary tooth, probably masking the effect of the permanent tooth agenesis, especially in younger children.⁷⁵ One of the optimum treatment standards in pediatric dentistry is the esthetic demand, which impacts on the child's OHRQoL, and subsequently the child's general health quality of life. Thus, it is beneficial to the dentist to identify the influence of esthetic restorations on the OHRQoL of preschool children.⁷⁶ The OHRQoL of preschool children treated with zirconia crowns was described as significantly better as compared to those who received resin-bonded composite strip crowns. An adapted and validated Early Childhood Oral Health Impact Scale (ECOHIS) questionnaire could be an excellent tool to distinguish children without agenesis from those with a moderate to high percentage of missing teeth, like it has been made for caries experience,⁷⁷ or to determine the impact of dental treatment on OHRQoL.⁷⁸ There is still no evidence of a long-term survival of mandibular 2pm, and to accurately answer the typical questions from the patient: "For how long can my primary tooth survive if we decide to leave it in situ?" or "Will it be healthy and functional?", is yet tricky.³⁸ Well-designed, longitudinal, prospective controlled studies comparing the benefits and risks of the interceptive extraction of the primary molars or preserving the primary molar as a substitute for the absent permanent tooth in children in the early mixed dentition are an emergent need.⁷⁹

Using video-sharing platforms and virtual social networks can be helpful to spread information among patients. Nevertheless, the information disseminated should be scrutinized and weighted with well-defined criteria,^{80,81} and healthcare professionals, academic institutions and professional organizations should direct patients to reliable and more authoritative information sources, allowing consumers to critically assimilate the information posted in order to make effective healthcare decisions.^{82,83}

Teledentistry for oral screening, especially in school-based programs, rural areas, and areas with limited access to care, could also be used to identify tooth agenesis. Teleconsultations are possible and valid,⁸⁴ if the business model and cost-effectiveness concerns related to the time spent, particularly in the context of developing countries, are taken into account, as the preferred way seems to be a video-conference, followed by a phone call.⁸⁵

Some of the cases of missing teeth are complex clinical situations that require treatment involving not only the dentist, but also other medical specialists, such as the internist, the neurologist, the psychiatrist, the endocrinologist, the cardiologist, and the dermatologist.⁶⁴

Considering all these concepts, with this study, we aimed to contribute to the understanding of the natural evolution of the second primary molar (2pm) in the population not selected according to the orthodontic issues, and to estimate the longevity of 2pm, given its root resorption, occlusal positioning and the behavior of the adjacent teeth, with the perspective of its preservation in the oral cavity as a lasting therapeutic option, but also bearing in mind that low-income countries have financial constraints regarding complex treatment, such as orthodontics or implant-supported crowns.

To frame our study theoretically, a mini-narrative review was done.

Material and methods

An observational, cross-sectional and retrospective study was developed by analyzing digital orthopantomograms from the clinical records of outpatients at the Dental Clinic of the University Institute of Health Sciences (IUCS)/CESPU, Gandra, Portugal, from 4 consecutive years (January 2014–December 2017).

The STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines for reporting observational studies were followed. The ethical approval was provided by the Ethics Committee at IUCS/CESPU.

The hypotheses formulated were: H_1 – the second primary molar (2pm) has root and occlusal conditions to preserve the space corresponding to the absent permanent tooth for at least 15 years; H_0 – the second primary molar (2pm) does not have root or occlusal conditions to preserve the space corresponding to the missing permanent tooth.

Study population and data collection

Based on a preliminary sample of 12,949 orthopantomograms, 6,001 (46.34%) from males and 6,948 (53.66%) from females, 61 patients – 25 (40.98%) males and 36 (59.02%) females, aged 7–36 years, with a mean age of 16.38 ± 7.96 years – were diagnosed simultaneously with M2P agenesis and the 2pm retention. The 3rd quadrant and the 4th quadrant (tooth 3.5 or 4.5) were registered separately.

Oligodontia, cleft palate, syndromic cases, bone defects, the evidence of surgery or extraction, trauma, fractures, or previous orthodontic treatment were excluded.

Error of the method

The orthopantomograms were acquired with a digital device (PaX-400; Vatech, Hwaseong, South Korea) and after standardized photographic printing, analyzed to determine which teeth were present, absent or extracted. The subsequent measurements were done with an ortho-

odontic ruler (Dentaurum, Ispringen, Germany), following the method of Odeh et al.⁸⁶ One investigator systematically observed all orthopantomograms, and a second one blindly and randomly followed half of the sample for calibration and to discuss possible doubts. An administrative employee blindly coded the orthopantomograms to avoid the examination bias. Afterward, the results of the examinations were sorted by groups for statistical comparisons.

Evaluation of the measurement error

In evaluating the intra-observer and inter-observer variability corresponding to the observations of the variables involved in this investigation, 13 randomly selected patients from the initial sample were considered. In the inter-observer variability study, the 13 individuals were evaluated by 2 independent observers. For assessing the intra-observer variability, the investigator performed measurements on the 13 patients on 2 occasions, with a 2-month interval. The variability was evaluated through the intraclass correlation coefficient (ICC) with the determination of the confidence interval (CI). Table 1 shows the mean (*M*) and standard deviation (*SD*) values with regard to the examined variables of a quantitative nature, and the respective ICCs assessed by the same investigator (Observer 1).

Similar mean values were observed at both time points. The ICC values were considered high (1 corresponds to a perfect agreement) and very close to each other, revealing a good agreement between the 2 observations for all quantitative variables.

The statistical values (*M* ± *SD*) to assess the inter-observer variability were calculated based on measurements from 2 different investigators (Observer 1 and Observer 2). They are shown in Table 2, together with the ICC values.

Similar mean values were observed for the 2 observers. The ICC values were high and very close to each other, verifying a good agreement for all quantitative variables and suggesting the reliability of the analyzed data.

Table 1. Intra-observer agreement of the variables under study

Variable	Observation 1 <i>M</i> ± <i>SD</i>	Observation 2 <i>M</i> ± <i>SD</i>	ICC (95% CI)
RR	0.36 ± 0.26	0.38 ± 0.30	0.950 (0.835–0.985)
Width X [mm]	13.31 ± 1.70	13.54 ± 1.20	0.935 (0.788–0.980)
Width Y [mm]	10.77 ± 2.17	10.84 ± 2.30	0.926 (0.759–0.978)
Infraocclusion [mm]	2.46 ± 1.07	2.67 ± 1.16	0.977 (0.924–0.993)

M – mean; *SD* – standard deviation; ICC – intraclass correlation coefficient; *CI* – confidence interval; RR – root resorption; width X – mesiodistal width of the second primary molar (2pm); width Y – distance between the mesial face of the first permanent molar and the distal face of the first primary molar or the first permanent premolar.

Table 2. Inter-observer agreement of the variables under study

Variable	Observer 1 <i>M</i> ± <i>SD</i>	Observer 2 <i>M</i> ± <i>SD</i>	ICC (95% CI)
RR	0.37 ± 0.26	0.38 ± 0.24	0.835 (0.460–0.950)
Width X [mm]	13.31 ± 1.70	13.63 ± 1.45	0.759 (0.345–0.920)
Width Y [mm]	10.77 ± 2.17	10.38 ± 1.81	0.926 (0.759–0.978)
Infraocclusion [mm]	2.46 ± 1.07	2.46 ± 1.05	0.978 (0.925–0.993)

Sample grouping

The groups were as follows: group 1 – the first permanent molar in occlusion (*n* = 23); and group 2 – the second permanent molar also in occlusion (*n* = 38). A subdivision was made to correlate root resorption (RR), width X, width Y, infraocclusion, and age.

Orthopantomography analysis

Methods and tools were defined as follows:

- the degree of RR, evaluated according to a 6-point scale (the Bjerkin and Bennett method)³⁸ (Fig. 1A), assessing the distal and mesial roots. The highest root value was scored for the tooth; scores 4, 5 or 6 (i.e., ¾ of the root or more resorbed) were considered as a poor root condition;
- infraocclusion (the distance from the occlusal plane to the occlusal surface of the 2pm in millimeters) (Kurof's method)⁸⁷ (Fig. 1B);
- width Y (the distance between the mesial face of the first permanent molar and the distal face of the first primary molar or the first permanent premolar in millimeters) (Fig. 1C); and
- width X (the mesiodistal width of the 2pm in millimeters) (Fig. 1D).

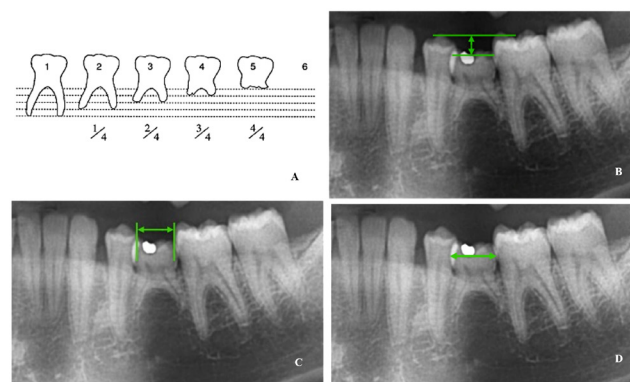


Fig. 1. A – different root resorption (RR) stages, measuring the quarters of each root (adapted from Bjerkin and Bennett (2000)³⁸); B – measurement of the primary tooth infraocclusion; C – measurement of width Y; D – measurement of width X

Statistical analysis

The descriptive data was presented as mean and standard deviation ($M \pm SD$), or as frequency and percentage (n (%)). The χ^2 test was used to assess the existence of dependence between 2 qualitative variables. The Monte Carlo simulation techniques were used whenever the applicability conditions of the χ^2 test were not met. Spearman's and/or Pearson's correlation coefficient was used to assess the degree of association between 2 variables (ordinal or continuous). Comparisons between groups, based on quantitative variables, were performed with the use of parametric tests whenever their applicability assumptions were satisfactory; otherwise, nonparametric alternatives were used. The Shapiro–Wilk test assessed the assumption of normality and Levene's test – the homogeneity of variance. A p -value ≤ 0.05 was considered statistically significant. Descriptive, graphical and inferential statistical analyses were performed using the IBM SPSS Statistics for Windows software, v.20.0 (IBM Corp., Armonk, USA).

Results

Group 1 presented a mean age significantly lower than group 2 (9.39 vs. 20.61 years) ($p < 0.001$).

The prevalence of M2P agenesis associated with the 2pm retention was 0.47% in the total sample, affecting tooth 4.5 in 50.8% ($n = 31$) and affecting tooth 3.5 in 49.2% ($n = 30$) of the cases. Inferential statistical analysis indicated that the percentage of patients affected by tooth 3.5 or 4.5 agenesis was not significantly different from 50.0%, so prevalence was similar in both quadrants.

The RR values were significantly different between the groups ($p = 0.001$). Group 1 had a higher frequency of low values, while group 2 had a higher frequency of values 0.50 (2/4 of RR) and 0.75 (3/4 of RR). Root resorption of the 2pm increased when the second permanent molar was also in occlusion, but it was impossible to detect its ending (Fig. 2A).

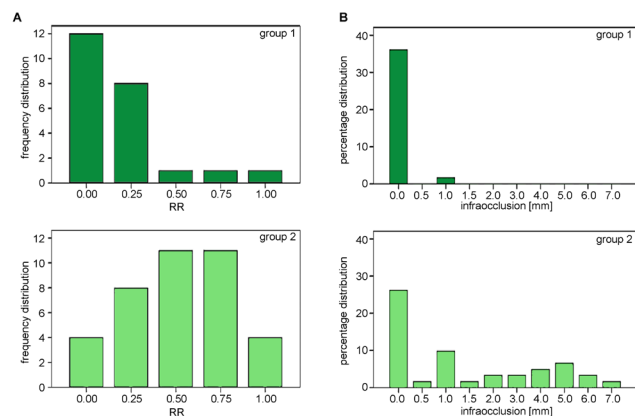


Fig. 2. A – distribution of root resorption (RR) according to group; B – distribution of infraocclusion according to group

Infraocclusion differed significantly between the groups ($p = 0.036$). The most frequent value was 0 mm (in occlusion) for both groups. In group 1, the values ranged from 0 mm to 1 mm, while in group 2 they ranged from 0 mm to 7 mm, being more often 0 mm or 1 mm, but increasing with age (Fig. 2B).

With the fundamental hypothesis being a zero correlation coefficient, the relationship between width X and width Y was compared among the groups. The correlation coefficients and p -values associated with the statistical test were calculated (Table 3). The dispersion diagram between width X and width Y according to group is displayed in Fig. 3.

The mean width X was significantly higher than the mean width Y in both groups, so the influence of the group on that difference was analyzed. We found a mean difference between width X and width Y of 2.09 mm in group 1 and of 2.77 mm in group 2. However, the equality between these 2 averages was not rejected ($p = 0.269$) (Table 4).

The correlation coefficients for the variables RR, width X, width Y, and infraocclusion with regard to age were calculated separately in the total sample, group 1 and group 2. Low correlation coefficients were found, significantly different from zero only for the whole sample. The strongest correlation with age was found for root resorption and infraocclusion. There was also a weak correlation between age and width Y, but still significantly different from zero (Table 5).

Table 3. Relationship between width X and width Y according to group

Statistics	Group 1	Group 2
r	0.408	-0.079
p-value	0.048*	0.639

* statistically significant.

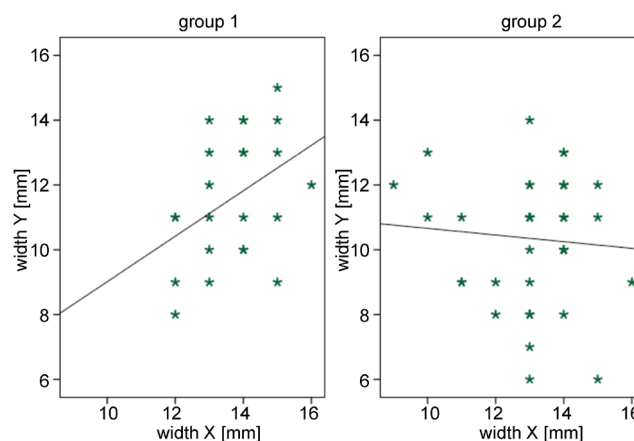


Fig. 3. Dispersion diagram between width X and width Y according to group

Table 4. Comparison between width X and width Y

Group	Width X [mm]	Width Y [mm]	p-value
Group 1	13.70 \pm 1.15	11.61 \pm 1.97	<0.001*
Group 2	13.11 \pm 1.97	10.34 \pm 1.94	<0.001*

Data presented as $M \pm SD$. * statistically significant.

Table 5. Correlation between the variables root resorption (RR), width X, width Y, and infraocclusion and age

Variable	Total sample		Group 1		Group 2	
	r	p-value	r	p-value	r	p-value
RR	0.408	0.001*	0.066	0.763	-0.112	0.504
Width X	-0.129	0.324	-0.087	0.694	-0.032	0.849
Width Y	-0.261	0.042*	-0.167	0.445	-0.045	0.790
Infraocclusion	0.483	<0.001*	-0.248	0.255	0.142	0.394

* statistically significant.

To confirm those results, age categorization for each group was done to determine how the mean values of RR, width X, width Y, and infraocclusion varied according to age subgroups.

The results are displayed in Tables 6,7 and Fig. 4. In group 1, the mean RR was similar in both age subgroups, slightly reducing with age. In group 2, the lowest mean RR value was observed for patients over 30, followed by those aged 21–25 years; for subgroup 26–30 years, the mean RR value was similar to those observed in the first 3 age subgroups. Comparing the groups, group 1 presented lower RR values.

In group 2, the mean infraocclusion was approx. zero for patients under 11 years of age, with a progressive increase up to 21–25 years, followed by a decrease with age. In group 1, an average infraocclusion was approx. zero in both age subgroups.

Regarding width X, in group 2, the subgroups up to 20 years and that of 26–30 years showed similar mean val-

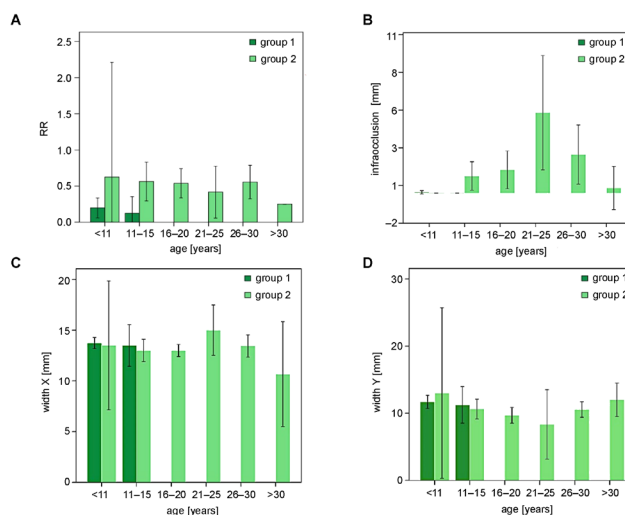


Fig. 4. A – root resorption (RR) according to group and age subgroups; B – infraocclusion according to group and age subgroups; C – width X according to group and age subgroups; D – width Y according to group and age subgroups

Table 6. Root resorption (RR), width X, width Y, and infraocclusion according to age subgroups in group 1 (n = 23)

Variable	Age [years]	
	<11 n = 19	11–15 n = 4
RR	0.20 ±0.23	0.13 ±0.14
Width X [mm]	13.74 ±1.15	13.50 ±1.29
Width Y [mm]	11.68 ±2.06	11.25 ±1.72
Infraocclusion [mm]	0.05 ±0.23	0.00

Data presented as M ±SD.

ues. The highest value was observed in subgroup 21–25 years and the lowest in patients over 30. In group 1, no differences were found. Globally, group 1 and group 2 did not differ.

Regarding width Y, in group 2, patients under 11 or over 30 showed the highest values, and subgroup 21–25 years showed the lowest value. In group 1, the mean width Y was nearly equal in both subgroups. Globally, group 1 and group 2 did not differ.

No significant movement of the adjacent teeth was observed in any of the groups or subgroups, so the vertical position of the teeth was apparently maintained.

Table 7. Root resorption (RR), width X, width Y, and infraocclusion according to age subgroups in group 2 (n = 38)

Variable	Age [years]					
	<11 n = 2	11–15 n = 8	16–20 n = 13	21–25 n = 3	26–30 n = 9	>30 n = 3
RR	0.63 ±0.18	0.56 ±0.32	0.54 ±0.34	0.42 ±0.14	0.56 ±0.30	0.25 ±0.00
Width X [mm]	13.50 ±0.71	13.00 ±1.31	13.00 ±1.00	15.00 ±1.00	13.44 ±2.56	10.67 ±2.08
Width Y [mm]	13.00 ±1.41	10.63 ±1.77	9.69 ±1.93	8.33 ±2.08	10.56 ±1.42	12.00 ±1.00
Infraocclusion [mm]	0.00	1.13 ±1.12	1.54 ±2.08	5.33 ±1.53	2.56 ±2.56	0.33 ±0.58

Data presented as M ±SD.

Discussion

The clinical decision to treat M2P agenesis associated with the retained 2pm is a challenging issue,⁶⁰ and the options to extract, thus allowing space closure, to prosthetically replace the missing tooth or to maintain the primary tooth in the arch implies reflection over various parameters, such as the health of the crown, pulp and the root of the primary tooth as well as of the surrounding bone,⁵⁰ the vertical position of the primary tooth relative to the occlusal plane; the presence of ankylosis of the primary tooth,⁶⁰ the patient's sagittal and vertical skeletal individual characteristics,^{62,88} the occlusal relationships and dental crowding, the patient's dental and chronological age,⁶² the presence of third molars, and the patient's preference for specific treatment or the expenditure of money.^{29,34,35}

Whenever the delayed exfoliation of the 2pm is detected, the diagnosis must necessarily be completed by the radiographic observation and verification of M2P agenesis,⁴⁷ as if it occurs, the therapeutic option is an urgent need, and in the majority of the cases, it is a complex therapy.

Based on the literature, globally, we can say that a healthy 2pm with no signs of ankylosis, no carious lesions or extensive restorations could be maintained with the expectation of extended survival. Nevertheless, the anteroposterior arch-length discrepancy must be controlled, sometimes by carrying out mesial and distal stripping, with a 2–3-millimeter reduction of the coronal length of the 2pm. One must be careful not to produce pulp lesions and be aware that such treatment is advisable mainly if later replacement with an implant is feasible. We must also be mindful that preserving the 2pm in function can have occlusal repercussions.

Also, in general, patients with minimal crowding, deep overbites, retrusive incisors, decreased lower facial heights, or flat mandibular planes may be candidates for no extraction, maintaining the 2pm for as long as possible. In the case of significant crowding, dental protrusion, minimal overbites or open bites, incisal inclination within a normal range, and increased lower facial heights, patients often benefit from extraction and space closure, but also with the extraction of the remaining 3 second premolars.⁸⁹ Meanwhile, based on clinical experience, we are confident that the premolar space closure with the use of an orthodontic device is more cost-effective, mainly if TADs are used to assist in space closure,⁷¹ often without the need for bone grafting, manual bone spreading⁹⁰ or osseodensification to increase ridge dimensions in a narrow alveolar ridge⁹¹ before the implant placement, or using a prosthetic restoration with inherent costly maintenance as compared to that of a natural tooth.

Bearing in mind those concepts, we chose patients from our University's Dental Clinic as the target population. The only initial requirement was having the digital

orthopantomography taken before the first consultation, available in the clinical records. In terms of selection criteria, the population differed from most of the populations from previous studies, as it was a raw population, i.e., it was not related to the orthodontics or various pediatric dentistry departments, so the patients had no prior diagnosis of an orthodontic issue or agenesis. This fact that could contribute to a certain bias.

Another peculiarity is that the average monthly income per capita of that population is less than half the country's mean reference value, which restricts onerous treatment, making the possibility of keeping the 2pm in function for a long time a socially fundamental therapeutic option.

Furthermore, since the clinical decision should be made as early as possible, ideally still in the early pediatric age (<9 years), we did not impose age restriction as an exclusion criterion and, by doing that, we expected to have a more realistic view of the natural evolution in cases not intervened.

In our selected sample, the mean age for group 1 was below that of group 2, as the established criterion for the eruption of molars was immediately an age constraint. Splitting the sample by the age of 11 had the purpose of separating the sample by the expected usual age of the exfoliation of the 2pm, possibly to identify differences in the biological behavior of a not yet exfoliated tooth and a retained one. Nevertheless, we must emphasize that our population comprised younger patients than the majority of previous studies, which is a pertinent issue if we assume that infraocclusion of the mandibular 2pm can be diagnosed since the age of 5 with a peak at 8–9 years,⁹² a statement that is inconsistent with our findings, as we found a close to 0 incidence below the age of 11 and a peak in the subgroup of 21–25 years.

A 1.44 times higher frequency of M2P agenesis was found in females, in accordance with another retrospective study,⁹³ but in conflict with one conducted on an Asian population,² possibly reflecting different selection criteria and the different genetic origin of the population.³⁰ In a Portuguese population of a similar origin, a study on the prevalence of the dental agenesis excluding third molars, conducted in 2005–2009, found a 1.30 times greater prevalence in females.³² In that study, the total prevalence of M2P agenesis was higher (6.0%) than ours, certainly due to the fact that we also required the presence of the retained 2pm. As back in 2005–2009, digital orthopantomography was not yet at our disposal, despite the temptation to enlarge our sample, that previous sample was not included in this study to avoid bias.

Although this is a cross-sectional study, RR, infraocclusion and width Y were correlated with age. The occasional high RR values correlated with M2P agenesis are not a surprise and were related to older patients, as resorption is expected to increase with age.³⁹ As group 2 had the second permanent molar in occlusion, we can extrapolate that only this group was older than 12 years. Consequent-

ly, we could compare our results with those of Bjerklin and Bennett,³⁸ who revealed a 60% mesial root resorption and a 46% distal root resorption at the age of 11–20 years for the 2pm, with a very slow and great inter-individual variation process.

The prevalence of M2P agenesis with the retained 2pm was similar in both quadrants. Arai found the same in the Japanese population,² but De Stefani et al. found a preponderance of left M2P agenesis in an Italian sample.¹ Symmetry may complicate the therapeutic decision in cases with no dental crowding (common in agenesis),⁴¹ deep overbites, or restrained lower facial development, as the normal mandibular development can be compromised bilaterally, advising the maintenance of the primary tooth.⁸⁹

Contrariwise, if the extraction of the 2pm is recommended due to decay or root resorption, it should be done as soon as possible to allow spontaneous effective space closure,²⁹ preventing the abnormal movement of the adjacent teeth or steeper occlusal curves, thus avoiding the need for later orthodontic treatment.^{94,95} It should be done soon after 9 years of age, but the first premolar should have at least half of the root length already developed.⁸⁹ Whenever possible, controlled mesial and distal stripping, followed by the hemi-sectioning of the 2pm before extraction should be performed, producing the controlled mesial movement of the first permanent molar.⁸⁹

The extraction must be performed with caution to maintain the cortical walls, especially in cases of ankylosis, as the alveolar ridge progressively loses width, mainly due to the loss of the buccal side of the ridge,⁶⁷ and if the extraction is performed after the eruption of the second permanent molar, space maintainers are not recommended, even if implants are planned. In such a situation, the drifting of the adjacent teeth should be allowed for some space closure, and the teeth should be posteriorly verticalized, recreating space for the implant, and thus maintaining the ridge.

A recent 3D finite element analysis found that the kind of occlusal forces influences the pattern of root resorption.⁴⁰ Other authors showed that the 2pm could remain stable without additional root resorption after 20 or up to 15 years after the exfoliation age.³⁹ Another retrospective radiographic study with patients aged 21–77 years found an insignificant reduction of the root length of all primary teeth, on average 0.16 mm over 5 years.⁵⁰ In our study, group 2 presented worse cases of root resorption, despite the most frequent values being 0.50–0.75, which is in line with Bjerklin and Bennett.³⁸

The mean infraocclusion was close to 0 mm, as in a study by Bjerklin et al.,²⁸ with a value of no more than 0.1 mm. However, Bjerklin and Bennett³⁸ previously concluded that 55% of patients with the retained 2pm had infraocclusion of a value far exceeding ours, probably due to the measurement reference points, necessarily modi-

fied with regard to the patient age, as observed in other studies.^{86,87} This aspect should be further explored, as the reference points used for the determination of infraocclusion have not been standardized.⁹⁶ In 2016, objective criteria for measuring infraocclusion with a high reproducibility of the results were described,⁹⁶ but their applicability to different age groups is yet to be proven.

Our findings regarding no gender prevalence and the lack of a significant association between infraocclusion and the arch side are compatible with a previous study, which further described the 2pm as the most infraoccluded tooth.⁸⁶ Another study, not requiring the retained 2pm, found a slight preponderance of bilateral agenesis and unilateral right-sided agenesis, and a significantly higher prevalence of the microdontia of maxillary lateral incisors.⁴³

Width Y remained stable throughout age, with no significant loss of space and no place for mesial movement. Our findings for groups 1 and 2 are compatible with data from other studies,⁹⁵ and are probably due to the close to 0 mm infraocclusion mean value. Although minimal, it must be monitored in some cases, as early infraocclusion is detrimental and leads to the tooth loss. Even so, paradoxically, teeth with short roots are more prone to be stable over time.³⁷

Maintaining the 2pm as a therapeutic option may compromise occlusion due to the unavoidable Bolton discrepancy caused by a larger mesiodistal size of the 2pm relative to its permanent successor.⁹⁵ To equate occlusal interference or reduce the occlusal surface width, it is advisable to diminish occlusal forces.⁴⁰

Given the possibility of temporomandibular joint dysfunctions and the desired age of agenesis diagnosis, despite MRI still being the gold standard for the identification of joint structures, the ultrasound scan should be considered, regardless of its lower diagnostic efficiency in evaluating the disk position during joint movements, due to some clinical advantages in terms of costs, accessibility and easier monitoring of young patients. Nevertheless, the obtained data must be corroborated by clinical and anamnestic data.^{97,98}

M2P agenesis with the retained 2pm is a challenge,^{95,99} with several issues to be considered, such as extracting or not, or re-anatomizing, restoring, or preserving the 2pm.^{37,40,92,99,100} Clues are scarce, as revealed by the search in the databases, as only one systematic review with a specific survival rate for the 2pm (83–93%) was found, and it was based on the data extracted from only 4 longitudinal observational studies with follow-ups of 5–15 years.¹⁰⁰

The prognostic factors are root resorption, infraocclusion, caries/restorations, and the periodontal status.¹⁰⁰ If ankylosis is present, the treatment plan is urgently required, and extraction/space closure, extraction/transplantation or extraction/prosthesis must be considered as the best plan,⁶¹ provided the loss of the alveolar crest is equated since the 3rd month after the extraction of the primary tooth.⁶⁷ Another concern is that M2P agenesis is

frequently associated with other tooth anomalies, even in non-syndromic cases, especially with the agenesis of third molars from the same quadrant, which may be found in 48% of patients.^{33,48} As a third molar should only be considered as missing after the age of 14, the decision to early extract the retained 2pm can be risky, since space closure will occur before it is absolutely certain that a third molar is present, leaving the possibility of the associated third molar agenesis open, with the consequent absence of a vertical stop to maxillary second molar.

We found that the age of 10–15 years and 21–25 years were critical phases for the loss of the 2pm. Surviving those phases with a favorable occlusal function boosts longevity, which could encourage research in populations far beyond the pediatric age.

Given our results, the hypothesis H_1 was accepted, and H_0 was rejected, as we found that the 2pm had the root and occlusal conditions to preserve the space for the corresponding absent M2P for at least 25 years, a finding beneath the interval found by Bjerklin et al. (16–30 years).²⁸

Longitudinal randomized clinical trials (RCTS) with the inter-study standardization of the evaluation criteria and well-defined clinical evaluation of the occlusion/function parameters are needed to calculate the real mean longevity of these second primary molars and support the general dentist, especially when there are no other reasons for carrying out orthodontic treatment.

Limitations

The retrospective design is a limitation of the present study. Nevertheless, the original sample was considerable in terms of size. The population studied originated from the general population and not from orthodontic or pediatric dentistry patients. The selected sample had no age restriction. Another limitation might be that there were more clinical records from female patients than from male patients due to the unbalanced gender ratio in dental clinics. Still, even so, we found a relatively higher prevalence of M2P agenesis with the retained 2pm in females than in males. Working with the data obtained from patients within an age window of 29 years (7–36 years) and a mean age of 16.38 years allowed drifting away from the mean expected period for the exfoliation of the primary molar, which was a positive factor in terms of reducing the possibility of biased results due to individual differences in the age of exfoliation.

Clinical considerations

Given the possible extended survival of the second primary molar, a well-documented no-intervention treatment must be weighted, mainly in cases without orthodontic issues or with financial restraints, as the second primary molar can survive for a similar or even longer period as compared to a prosthetic option.

Conclusions

There is a good prognosis for the survival of the second primary molar when it remains beyond the average age of its exfoliation in cases of second premolar agenesis. In our study, we showed that it can replace the absent permanent premolar up to 36 years of age (the oldest patient found with both second premolar agenesis and the second primary molar retention).

Mandibular second premolar agenesis occurs with the retention of the mandibular second primary molar beyond the age of 25. If so, it will probably last for a long time, as root resorption decreases after that age.

The loss of space caused by the second primary molar infraocclusion is not a frequent problem, as infraocclusion is not significant in most cases, with higher values found in the oldest adult patients.

Ethics approval and consent to participate

The ethical approval was provided by the Ethics Committee at the University Institute of Health Sciences (IUCS)/CESPU, Gandra, Portugal.

Data availability

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

Consent for publication

Not applicable.

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Surgical-anatomical evaluation of mandibular premolars by CBCT among the Italian population

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Abstract

Background. The thorough knowledge of the anatomy of mandibular premolars is an essential factor for a correct approach to endodontic treatment, concerning both non-surgical and surgical treatment.

Objectives. Since there is no data on the Italian population in this context, the aim of this study was to evaluate, from a surgical perspective, the anatomy of mandibular premolars among the Italian population through a cone-beam computed tomography (CBCT) analysis, considering the morphology of their root canals according to Vertucci's classification and the prospect of their apices with regard to the vestibular bone plate and the proximity to the inferior alveolar nerve and the mental foramen as well as to evaluate the most appropriate distance from the apex in the radicular resection (3 mm or 5 mm).

Material and methods. At total of 492 CBCT acquisitions (from 246 males and 246 females) were included retrospectively, evaluating 720 mandibular premolars. Age, gender, the tooth position in relation to the vestibular plate, the number of roots, the tooth length and the root length, the number of canals, the configuration of the root canal system according to Vertucci's criteria, C-shaped canals, the distance from the cemento-enamel junction (CEJ) to the canal bifurcation, the number of apical foramina, and the distance from the apex to the inferior alveolar nerve or the mental foramen were evaluated.

Results. The study highlighted the frequent anatomic variability of the root canal system. It found the presence of 1 root for first and second premolars in 97% and 99% of cases, of 2 roots in 2.7% and 0.7% of cases, and of 3 roots in 0.3% and 0.3% cases, respectively. In 92% of cases, the mental foramen was located below a second premolar, or between a first premolar and a second premolar; only in 8% of cases, it was located close to a first premolar, but never as close as to a second premolar.

Conclusions. Mandibular premolars show a truly surprising anatomical variability, especially for mandibular first premolars, which therefore requires adequate radiographic planning before providing any endodontic treatment, or especially endodontic retreatment or endodontic surgery. Taking into account proximity to the inferior alveolar nerve and the mental foramen, any surgical approach must be carefully planned. Frequently, a two-dimensional (2D) radiographic examination is not sufficient to fully understand the anatomical variability of these teeth.

Keywords: endodontics, CBCT, mandibular premolars, endodontic surgery, inferior alveolar nerve

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Introduction

The complete understanding of the anatomy of mandibular premolars represents a major challenge for the endodontic practitioner, since the correct interpretation of the root canal system in terms of canal morphology and number of canals is fundamental for the successful outcome of endodontic therapies.¹ Too frequently mandibular premolars, but especially mandibular first premolars, are thought to have a single root with a single canal. This belief leads to accessing cavities in an extremely conservative way, which does not help in locating any other possible canal orifice, with a rate of around 10% of premolar teeth where one or more canals have not been instrumented and a prevalence of consequent apical periodontitis of around 50%.^{2,3} The problem is even greater considering that these teeth often have anatomy that is difficult to interpret, especially in their middle and apical thirds, which makes the outcome of endodontic treatment even more uncertain due to the lack of instrumentation and/or irrigation.^{4,5}

Technological development has led to the improvement of the sensitivity of radiographic diagnostic examinations and considerably increased the understanding of the endodontic root canal system. Hence, in the last years, the international research has focused on how frequently the endodontic anatomy, in particular of some teeth, is underestimated and not recognized.^{6–10}

The risk of performing endodontic treatment without having understood the dental anatomy represents a condition of extreme danger of overloading the rotating endodontic instruments with torsional stress and cyclic fatigue, which may lead to their intracanal separation.^{1,11} Thus, the knowledge of the root canal anatomy is an essential factor for the choice of rotary instruments to be used during an endodontic therapy.^{12,13}

As compared to other teeth, premolars show high heterogeneity in terms of anatomical features, which is related to several factors, including age, sex and ethnicity.^{2,4}

Although intraoral radiographs are considered as the imaging modality of choice in the evaluation of the endodontic patient, performing cone-beam computed tomography (CBCT) is absolutely recommended when the diagnosis cannot be confidently determined in order to exclude any type of risk during the orthograde root canal therapy. Instead, as provided in the position statement of the American Association of Endodontists (AAE) and the American Academy of Oral and Maxillofacial Radiology (AAOMR), limited field-of-view (FOV) CBCT should be considered as the imaging modality of choice for pre-surgical treatment planning in order to localize root apices and to evaluate their proximity to the adjacent anatomical structures.¹⁴ The proximity of the apices of mandibular premolars to the inferior alveolar nerve represents a very important risk factor with regard to the root canal therapy in its instrumentation, irrigation, and especially obturation

phases, as there might occur transient or permanent lesions to the inferior alveolar nerve.^{15–17} The foregoing is even more valid when surgical endodontic treatment has to be performed to resect the terminal part of the root of a mandibular premolar, recommended at 3 mm from the apex.¹⁸ From this point of view, it also becomes necessary to analyze the perspective of root apices with regard to the vestibular bone plate in order to carefully plan a surgical approach, considering their proximity to the inferior alveolar nerve or the mental foramen and its anterior loop.^{14,18}

Therefore, since there is no data regarding the Italian population in this context, the objective of this study was to analyze the anatomy of mandibular premolars with Vertucci's classification⁴ using CBCT, to evaluate the prospect of their apices with regard to the vestibular bone plate and the proximity to the inferior alveolar nerve and the mental foramen, and to evaluate the most appropriate distance from the apex in the radicular resection (3 mm or 5 mm) in order to establish the most predictable approach.

Material and methods

The present CBCT study was conducted retrospectively, and all evaluations were conducted in accordance with the Declaration of Helsinki. Furthermore, the study was approved by the institutional ethics committee at the Department of Oral and Maxillofacial Sciences of the Sapienza University of Rome, Italy (protocol No. 528/17). All the analyzed CBCT scans had been previously acquired for reasons other than this research, such as oral surgery planning, the extractions of third lower and upper molars, and the implant placement. The CBCT scans were taken with the Orthophos SL 3D imaging unit (Dentsply Sirona, Wals bei Salzburg, Austria) set at 500 ms, 7 mA, 85 kV, 8 cm × 8 cm FOV, and a resolution of 0.16 mm; the software used for the analysis of the images was Sidexis Galileos Implant, v. 9.1 (Dentsply Sirona).

The inclusion criteria for considering mandibular premolars were as follows:

- the presence of at least 1 mandibular first or second premolar;
- complete root formation;
- no resorption signs;
- no previous endodontic treatment;
- no posts or coronal restorations;
- no root canal calcification – root canals had to be visible from the pulp chamber to their apical part;
- not having any condition that could limit the possibility of identifying structures being the object of the study, such as neoplasms, cysts, large peri-radicular lesions, artifacts, or internal or external root resorption;
- age over 18 years; and
- belonging to the Italian population.

The evaluated variables were as follows:

- age;
- gender;
- the tooth position in relation to the vestibular plate (Fig. 1);
- the number of roots;
- the tooth length and the root length;
- the number of canals;
- the configuration of the root canal system according to Vertucci's criteria (Fig. 2)
- C-shaped canals;
- the distance from the cementoenamel junction (CEJ) to the canal bifurcation (if present);
- the number of apical foramina; and
- the distance from the apex to the inferior alveolar nerve or the mental foramen (Fig. 3).

Based on the selection criteria, 720 mandibular premolars were included in the study (380 mandibular first premolars and 340 mandibular second premolars) after 492 CBCT acquisitions (from 246 males and 246 females).

Before proceeding to any surgical evaluation, the endodontic anatomy of these mandibular premolars was studied, bearing in mind the classification proposed by Vertucci.

First of all, the number of roots was considered, which is fundamental for evaluating a possible surgical approach. The root canal system was established following the indications provided in the Vertucci's classification: type I (1-1); type II (2-1); type III (1-2-1); type IV (2-2); type V (1-2); type VI (2-1-2); type VII (1-2-1-2); type VIII (3-3), and other possible configurations, such as C-shaped canals.⁴

In order to simulate the execution of the apical resection at 90°, following the recommendations of the 'modern technique' proposed by Kim and Kratchman,¹⁹ a cut at 3 mm from the apex was simulated. The decision

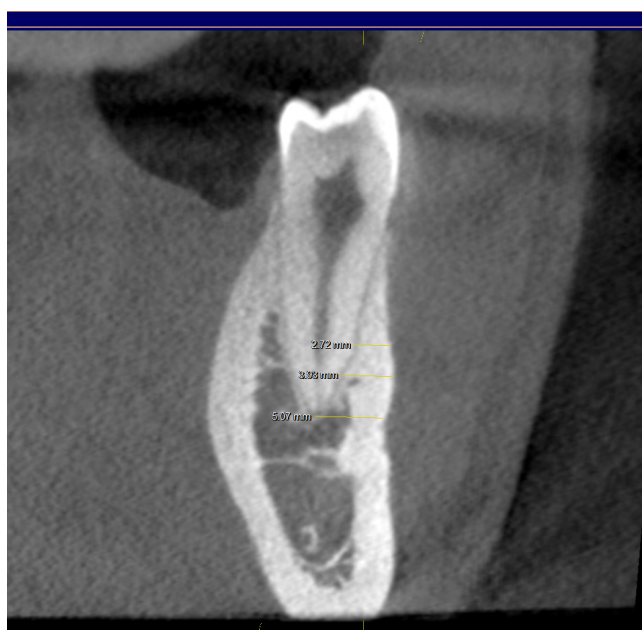


Fig. 1. Example of the measurements of the distance from the apex to the vestibular cortical plate at 0 mm, 3 mm and 5 mm from the apex

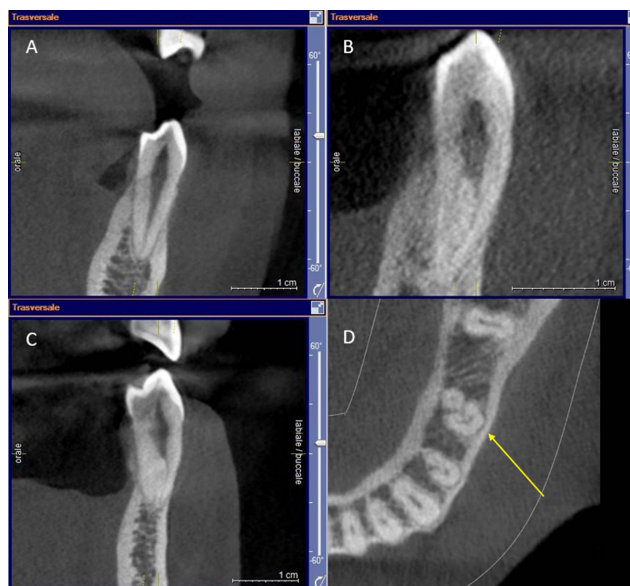


Fig. 2. Examples of root canal configuration according to Vertucci's classification A – type I; B – type III; C – type V; D – C-shaped canal (yellow arrow) in a transversal view.



Fig. 3. Example of the measurement of the distance from the apex to the inferior alveolar nerve or the mental foramen

where to perform the apical resection was complicated by the fact that in many cases, only resection at 5 mm from the apex reveals the root canal anatomy that is more easily manageable in retrograde surgical endodontics.

The following characteristics were studied at the levels of 3 mm and 5 mm:

- the number of root canals present;
- root canal configuration;
- apical foramina (Fig. 4);
- the distance from the apex to the vestibular plate (at 0 mm, 3 mm and 5 mm from the apex); and
- the distance from the apex to the inferior alveolar nerve or the mental foramen.

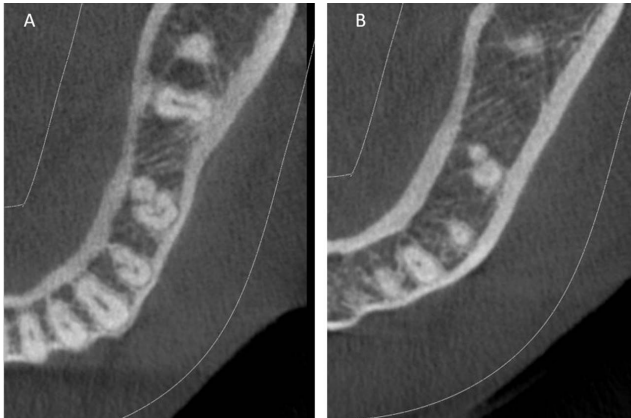


Fig. 4. Transversal view of a root canal at 3 mm (A) and at the apex (B), showing the number of apical foramina

All measurements were performed by 2 authors (R.R and A.Z.), calibrated after performing the analysis of the first 50 CBCT scans together.

Table 1. Root canal configuration

Vertucci's type	First premolars <i>n</i> = 380	Second premolars <i>n</i> = 340
I	281 (73.95)	320 (94.12)
II	15 (3.95)	4 (1.18)
III	4 (1.05)	2 (0.59)
IV	61 (16.05)	9 (2.65)
V	15 (3.95)	4 (1.18)
VI	0 (0.00)	0 (0.00)
VII	0 (0.00)	0 (0.00)
VIII	2 (0.53)	0 (0.00)
Other	2 (0.53)	1 (0.29)

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

Table 2. Number of apical foramina

No. of apical foramina	First premolars <i>n</i> = 380	Second premolars <i>n</i> = 340
1	300 (78.95)	325 (95.59)
2	76 (20.00)	14 (4.12)
>2	4 (1.05)	1 (0.29)

Data presented *n* (%).

Table 3. Distance between the radiographic apex and the mental foramen [mm]

Measurement point	First premolars <i>n</i> = 380		Second premolars <i>n</i> = 340	
	A-C	M-D	A-C	M-D
0 mm	3.40 ± 1.80	4.12 ± 1.94	3.44 ± 1.60	1.20 ± 1.12
3 mm	4.12 ± 1.84	4.32 ± 2.12	4.15 ± 2.25	1.25 ± 1.02
5 mm	5.87 ± 2.81	4.47 ± 2.18	5.12 ± 1.98	1.34 ± 1.12

Data presented as mean ± standard deviation (*M* ± *SD*). A-C – apicocoronal; M-D – mesiodistal.

Statistical analysis

The statistical analysis of the data was performed using the IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA).

Descriptive statistics were presented as mean and standard deviation (*M* ± *SD*), and frequencies for categorical variables. For continuous, normally distributed variables, differences between the various groups were calculated with Student's *t* test. The linear and logistic regression method was used for each study variable (the independent variable) in relation to any primary outcome (the dependent variable). The level of significance was set at *p* = 0.05.²⁰

Results

The study found the presence of 1 root for first and second premolars in 97% and 99% of cases, of 2 roots in 2.7% and 0.7% of cases, and of 3 roots in 0.3% and 0.3% cases, respectively. All the registered data was stratified according to gender (male and female) and age (3 age groups of equal size: 18–43 years; 44–69 years; and ≥70 years).

In 92% of cases, the mental foramen was located below a second premolar, or between a first premolar and a second premolar; only in 8% of cases, it was located close to a first premolar, but never as close as to a second premolar.

The root canal anatomy according to Vertucci's types is presented in Table 1.

After this evaluation, the number of orifices the premolars had at their apices was studied; the results are presented in Table 2.

The relationship between the mandibular premolars and the mental foramen has been resumed in Table 3, whilst the perspective of their apices with regard to the vestibular plate is shown in Table 4.

The evaluation of possible endodontic anatomy that could be found in apical resection at 3 mm or 5 mm was carried out. The results for the apical resection at 3 mm are summarized in Table 5, while those for the apical resection at 5 mm in Table 6.

When major root resection is performed, it is necessary to evaluate the amount of residual bone in order to guarantee the stability of the tooth required to withstand

the chewing loads and to restore the tooth correctly. Precisely in this regard, and considering the different age groups of the patients included in the study, the length of the tooth was measured, taking into account also the partial length of its components, as well as the amount of the residual supporting bone. The results of dental measurements are summarized in Table 7; bone dimensions according to age are presented in Table 8.

Table 4. Distance between the radiographic apex and the vestibular plate [mm]

Measurement point	First premolars <i>n</i> = 380	Second premolars <i>n</i> = 340
0 mm	3.84 ±1.60	4.02 ±1.02
3 mm	3.45 ±1.40	3.75 ±1.24
5 mm	3.12 ±1.24	3.66 ±1.26

Data presented as $M \pm SD$.

Table 5. Number of root canals in the simulation of resection at 3 mm

No. of canals	First premolars <i>n</i> = 380	Second premolars <i>n</i> = 340
1	300 (78.95)	325 (95.59)
2	76 (20.00)	14 (4.12)
>2	4 (1.05)	1 (0.29)

Data presented *n* (%).

Table 6. Number of root canals in the simulation of resection at 5 mm

No. of canals	First premolars <i>n</i> = 380	Second premolars <i>n</i> = 340
1	315 (82.89)	325 (95.59)
2	61 (16.05)	10 (2.94)
>2	4 (1.05)	5 (1.47)

Data presented *n* (%).

Table 7. Tooth measurements [mm]

Teeth	Distance from the occlusal plane to CEJ	Distance from CEJ to the apex	Distance from CEJ to the canal bifurcation	Tooth length
First premolars <i>n</i> = 380	6.5 ±1.1	15.0 ±2.4	3.0 ±1.1	21.0 ±1.2
Second premolars <i>n</i> = 340	6.7 ±1.4	16.2 ±2.2	2.4 ±1.9	21.8 ±1.9

Data presented as $M \pm SD$. CEJ – cementoenamel junction.

Table 8. Bone level measurements (from the apex to the crestal bone) [mm]

Teeth	Age groups		
	18–43 years	44–69 years	≥70 years
First premolars <i>n</i> = 380	15.1 ±2.2	13.1 ±1.2	11.4 ±1.1
Second premolars <i>n</i> = 340	16.3 ±2.1	14.8 ±2.1	12.1 ±1.8

Data presented as $M \pm SD$.

Discussion

According to the results of the present study, it can be clearly stated that mandibular premolars are characterized by extremely variable anatomy. As previously pointed out by other authors, this anatomic heterogeneity should be considered while planning both non-surgical and surgical endodontic treatment.^{21–23} Furthermore, the results of this study clearly show the relationships between mandibular premolars and the nearby anatomical structures, underlining the importance of careful preoperative evaluation. This variability represents an important risk factor for the success of both orthograde endodontic treatment and any surgical approaches carried out to resolve non-surgical endodontic failure. In cases of inexplicable endodontic failure with a doubtful diagnosis, it is absolutely necessary to investigate through three-dimensional (3D) diagnostic imaging possible anatomical variations that may have influenced the outcome of the primary treatment, and consider and plan the subsequent surgical approach.¹⁴

The inclusion of patients of exclusively Italian origin does not allow us to affirm the variability between ethnic groups, but still offers the possibility of better assessing, excluding this variable, differences between genders and changes that occur with age. However, a comparison between ethnic groups can be made, considering the data obtained by other researchers (Table 9).

The results of this study are consistent with most of the published research listed in Table 9, with the most common anatomical configuration according to Vertucci's classification being type I (73.95% for mandibular first premolars and 94.12% for mandibular second premolars), followed by type IV (16.05% for mandibular first premolars and 2.65% for mandibular second premolars) (Table 1).

The study of the abovementioned characteristics by means of CBCT with a wide FOV certainly might be related to a greater risk of error as compared to the analysis of the extracted teeth, but in the case of artifacts that made the evaluation difficult, the affected teeth were not included, following the study inclusion criteria.

Considering the number of roots, particular attention must be paid to mandibular first premolars, since in the case of the presence of several roots, the root canal anatomy is often extremely complex (Fig. 4). Even if extremely rare, however, there could be a possibility of finding three-rooted teeth, especially in their apical portion.

In 92% of cases, the mental foramen was located below a second premolar, or between a first premolar and a second premolar; only in 8% of cases, it was located close to a first premolar, but never as close as to a second premolar. From this point of view, considering the extreme proximity of a second premolar to the inferior alveolar nerve or the mental foramen (Fig. 3), not only is an endodontic surgical approach more difficult, but also

Table 9. Overview of the incidences of some documented ethnic and population variations in the anatomy of mandibular first and second premolars

Study	Population	Sample size	Tooth	Root canal configuration according to Vertucci's types									
				I [%]	II [%]	III [%]	IV [%]	V [%]	VI [%]	VII [%]	VIII [%]	other [%]	
Vertucci ²¹ 1978	not specified	400	mandibular first premolar	70.0	4.0	1.5	24.0	0.5	0	0	0	0	
		400	mandibular second premolar	97.5	0	0	2.5	0	0	0	0	0	
Yang et al. ²⁸ 2013	Chinese subpopulation	440	mandibular first premolar	76.14	3.41	2.73	6.59	9.32	0	0	0.68	1.14	
Wu et al. ²⁹ 2020	Chinese subpopulation	1,296	mandibular first premolar	81.0	0	3.0	0	12.1	0	0	0.5	3.0	
Ok et al. ³⁰ 2014	Turkish population	1,471	mandibular first premolar	92.8	0.3	1.0	3.4	4.4	0	0	1.0	–	
		1,345	mandibular second premolar	98.5	0.1	0.1	0.9	0.5	0	0	0.2	–	
Alfawaz et al. ³¹ 2019	Saudi population	391	mandibular first premolar	88.0	3.6	3.1	2.0	1.5	0.3	0	1.5	–	
		343	mandibular second premolar	90.1	4.4	0.3	2.6	0.9	0	0	1.7	–	
Bulut et al. ³² 2015	Turkish population	585	mandibular first premolar	94.20	0.64	1.12	0.80	3.24	0	0	0	–	
		549	mandibular second premolar	98.9	0.2	0.4	0	0.5	0	0	0	–	
Awawdeh and Al-Qudah ³³ 2008	Jordanian population	500	mandibular first premolar	58.2	4.8	1.4	14.4	16.8	0.8	1.0	0	2.6	
		400	mandibular second premolar	78.0	3.8	0.1	5.5	12.3	0	0	0	0.5	
Baisden et al. ³⁴ 1992	USA population	500	mandibular first premolar	76	0	0	24	0	0	0	0	0	
Sandhya et al. ³⁵ 2010	Indian population	100	mandibular first premolar	80	9	3	2	4	0	0	0	2	
Velmurugan and Sandhya ³⁶ 2009	Indian population	100	mandibular first premolar	72	6	3	11	8	0	0	0	0	
Iyer et al. ³⁷ 2006	Chennai population	2,000	mandibular first and second premolars	75.4	1.0	0	20.8	2.4	0	0	0.4	0	
Parekh et al. ³⁸ 2011	Indian population	40	mandibular first premolar	50.0	5.0	5.0	25.0	12.5	2.5	0	0	0	
		40	mandibular second premolar	80.0	0	0	2.5	17.5	0	0	0	0	
Jain and Bahuguna ³⁹ 2011	Gujarati population	138	mandibular first premolar	67.4	8.0	3.7	3.9	16.4	0.7	0	0	0	
Sikri and Sikri ⁴⁰ 1994	Indian population	112	mandibular first premolar	82	9	3	2	4	0	0	0	0	
		96	mandibular second premolar	82	9	3	2	4	0	0	0	0	
Walker ⁴¹ 1988	Chinese population	100	mandibular first premolar	78	6	6	10	0	0	0	0		
Liu et al. ⁴² 2013	Chinese population	115	mandibular first premolar	65.2	0	2.6	0	21.6	0	0.9	0	0	
Khedmat et al. ⁴³ 2010	Iranian population	217	mandibular first premolar	90.0	1.8	3.2	0.9	4.1	0	0	0	0	
Rahimi et al. ⁴⁴ 2007	Iranian population	163	mandibular first premolar	70.6	1.9	3.8	3.8	16.9	1.2	0.6	0	1.2	
		103	mandibular second premolar	76.3	7.9	9.9	5.9	0	0	0	0	0	
Sert et al. ⁴⁵ 2004	Turkish population	200	mandibular first premolar	60.6	18.5	10.5	7.0	2.5	0	0	1.0	0	
		200	mandibular second premolar	71.0	7.0	3.5	9.0	7.0	1.5	1.0	0	0	
Calışkan et al. ⁴⁶ 1995	Turkish population	100	mandibular first premolar	64.15	7.55	3.77	7.55	9.43	1.89	0	5.66	0	
		100	mandibular second premolar	93.62	0	0	0	6.38	0	0	0	0	
Algarni et al. ⁴⁷ 2021	Saudi population	216	mandibular first premolar	68.50	11.10	6.01	1.38	12.10	0.92	0	0	0	
Dou et al. ⁴⁸ 2017	Chinese population	178	mandibular first premolar	62.04	1.12	9.67	0.56	16.91	0	0	1.12	9.36	

in the orthograde root canal therapy, despite the fact that the anatomy of a second premolar is much simpler than in the case of a first premolar, a lot of attention must be paid to avoid over-instrumentation, the extrusions of irrigants or the overfilling of the canal.

Taking into account the close proximity of the apices of premolars, in particular of mandibular second premolars, to the inferior alveolar nerve and the mental foramen, while planning a surgical endodontic operation, it is necessary to consider the possibility of using computer-aided navigation (static and dynamic) that allows minimally invasive interventions, at the same time preserving vital structures.^{24–26} Furthermore, proceeding with a piezoelectric system reduces the possibility of transient or permanent damage to the alveolar nerve, and this procedure is recommended.²⁷

On the other hand, having regard to the reduced distance between these teeth and the buccal plate, it is possible to perform an endodontic surgical procedure in a fairly easy way. Great attention must be paid to the design of the flap and to the isolation of the mental nerve from where it passes through the soft tissues. Furthermore, an osteotomic approach with static guidance or dynamic navigation, in association with a piezoelectric system, considerably reduces the risk of any nerve injury.

In the present study, the occurrence of C-shaped canals was not significant in the population, similarly to Vertucci's type VIII configuration (Table 1).

Conclusions

Despite the limitations of this study, mandibular premolars show a truly surprising anatomical variability, which therefore requires adequate radiographic planning before providing any endodontic treatment, or especially endodontic retreatment or endodontic surgery. Moreover, taking into account proximity to the inferior alveolar nerve and the mental foramen, any surgical approach must be carefully planned. Frequently, a two-dimensional (2D) radiographic examination is not sufficient to fully understand the anatomical variability of these teeth.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. The study was approved by the institutional ethics committee at the Department of Oral and Maxillofacial Sciences of the Sapienza University of Rome, Italy (protocol No. 528/17).

Data availability

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

Consent for publication

Not applicable.

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Comparison of the effectiveness of 38% silver diamine fluoride and atraumatic restorative treatment for treating dental caries in a school setting: A randomized clinical trial

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Abstract

Background. Dental caries is an infectious microbial disease of the teeth, affecting mostly young children. It is caused by the interplay of numerous factors that are more common in susceptible and lower-income groups. Therefore, there is a dire need for a simple yet effective method of treating dental caries.

Objectives. The aim of the study was to compare the effectiveness of 38% silver diamine fluoride (SDF) and atraumatic restorative treatment (ART) in the treatment of dental caries in a school setting.

Material and methods. A randomized clinical trial (RCT) using a parallel-group design was conducted among schoolchildren aged 6–12 years. Oral hygiene practices and sugar exposure were recorded at baseline. In the schoolchildren, 190 dental caries lesions were restored using 38% SDF (group 1) or ART (group 2). The χ^2 test and the Kaplan–Meier survival analysis were done to assess the 2 treatment modalities. The RCT was carried out and reported in accordance with the CONSORT (Consolidated Standards of Reporting Trials) checklist.

Results. A total of 190 children fulfilled the inclusion criteria and the mean age of the study population was 9.3 ± 1.3 years. The majority of the participants brushed their teeth once daily, using a toothbrush, rinsed their mouth after meals and had optimal sugar exposure. In measuring the outcome of the interventions after 9 months, 58.9% of the participants from group 1 had their restorations intact as compared to 47.8% in group 2, and this was found to be statistically significant ($p = 0.004$). The survival analysis using the log rank test showed 38% SDF to be better in comparison with ART.

Conclusions. This study indicated that SDF was better at arresting caries, and hence it can be considered a viable treatment option in areas with limited access to oral healthcare.

Keywords: dental caries, silver diamine fluoride, dental atraumatic restorative treatment, dental care for children

Introduction

Dental caries is an infectious microbial disease of the teeth, affecting mostly young children. It is caused by the interplay of numerous factors, and is more common in susceptible and lower-income groups. Especially in areas with limited access to dental services, the treatment of dental caries is a serious concern. Untreated dental caries has a negative influence on self-esteem, the intake of food and sleep, and it also hampers daily activities.¹ Therefore, there is a dire need for a simple yet effective method of treating dental caries. Usually, the treatment of caries requires technologically advanced dental equipment as well as a skilled and well-trained professional, thus making it an expensive treatment modality.¹ Developing and underdeveloped countries present with unmet needs due to the underutilization of the available services. Moreover, carrying out the treatment procedures poses a challenge with regard to children due to their limited adaptive capability.²

The use of rotary instruments causes vibrations and noise, and increases the temperature of the cut surface, raising the anxiety of an already distressed pediatric patient. It also poses the risk of pulpal exposure, weakening the tooth structure and postoperative pulpal pain. Due to the scarcity of equipment and trained manpower in most underdeveloped or developing regions, the conventional restorative dentistry is not a viable treatment option.³

Atraumatic restorative treatment (ART) came into picture in 1985 to surpass the shortcomings of the conventional restorative treatment, specifically targeting those who did not have access to dental care.^{3,4} Atraumatic restorative treatment involves the excavation of carious tissue only with the use of hand instruments, thereby foregoing the use of electrically operated equipment, followed by the insertion of a filling material, such as glass ionomer cement (GIC), resin-modified GIC (RMGIC), composite resins, and compomers.^{3,5} It is a biologically favorable approach with the conservation of the tooth structure by removing only the demineralized dentin while providing restorative care away from the traditional dental set-up, thus lowering the cost of dental care.^{6–8}

However, the provision of ART also requires the participation of skilled and trained manpower, which is still a challenge in areas with limited access to dental care. In recent times, the use of minimally invasive dentistry and caries-arresting treatment has gained worldwide acceptance. For the remineralization of carious lesions, fluorides have been used extensively. Silver diamine fluoride (SDF) with the molecular formula $\text{Ag}(\text{NH}_3)_2\text{F}$ was approved by the US Food and Drug Administration (FDA) in 2014 and since then, it has been used as an effective caries-arresting material.

Different studies conducted by Chu and Lo,⁹ Llodra et al.¹⁰ and Yee et al.¹¹ found that SDF could serve

as a substitute for restorations that require technique-sensitive procedures, and could effectively arrest caries. In a clinical trial conducted by Zhi et al., it was observed that SDF arrested 70% of carious lesions in the primary anterior teeth of Chinese preschool children.¹ The application of SDF was considered to be painless, comfortable and acceptable in taste by the parents of children in a study conducted by Clemens et al.¹² The use of SDF offers a dual advantage of remineralization and antimicrobial effect due to the fluoride and silver content, respectively, without the need of using instruments for the excavation of carious tissue.

However, the use of SDF causes the discoloration of the teeth, which may not be esthetically pleasing. This warrants a search for an effective restorative material that can enhance the cosmetic appearance. Glass ionomer cement can be used, as it is tooth-colored and contains a high concentration of fluoride ions, which aids in arresting active caries by remineralizing carious lesions and preventing further caries development.

Therefore, the present study aimed to compare SDF with ART as the positive control. The null hypothesis assumed that there would be no difference in the effectiveness of 38% SDF and ART in terms of arresting dental caries in schoolchildren.

Material and methods

A randomized parallel-arm clinical trial was carried out to compare the effectiveness of 38% SDF and ART in the treatment of dental caries. The trial was registered at Clinical Trials Registry – India (www.ctri.nic.in) (REF/2020/06/034629).

Ethical consideration

Informed written consent was obtained from the parents before clinical examinations and verbal assent from the children at the time of the procedure. The Ethical Review Board of the Institute of Medical Sciences and SUM Hospital at Siksha 'O' Anusandhan Deemed to be University, Bhubaneswar, India, granted the ethical clearance for the study (ref. No. DMR/IMS.SH/SOA/180316).

Study population

The study was conducted in the public schools belonging to the district of Khordha, Odisha, India. Schoolchildren aged 6–12 years were considered for the study, as dental caries prevalence is usually higher in this age group.^{13,14} The sample size was calculated after a pilot study on 10 patients. Using the G*Power statistical software, v. 3.1.9.4 (<https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower>), at a power of 90% with an effect size

$d = 0.5$ and a two-tailed type I error of 0.05, the sample size was set at 172 dental caries lesions, with 86 lesions in group 1 and group 2 each. The sample size was increased by 10% to 190 to account for any loss due to follow-up/attrition.¹⁵

Children with a minimum of 1 carious lesion, code V and VI, not involving the pulp, according to International Caries Detection and Assessment System (ICDAS), in any of the completely erupted permanent molars were recruited to the study.¹⁶ Only occlusal lesions were included. The information regarding SDF and ART was given to the parents, and a written consent was obtained from them. The children were also explained the procedures and their verbal consent was sought. Those children who did not fulfil the age criteria or presented with pain, tooth mobility, signs of pulpal involvement, or the destruction of more than $\frac{1}{3}$ of the crown were excluded.

Study design

The study was conducted in 3 phases over a period of 11 months. It was a prospective parallel randomized clinical trial (RCT) with a follow-up at 3, 6 and 9 months. The study participants were randomly allocated into 2 groups according to a sequence of random numbers, which was generated using Microsoft® Excel, v. 2007 (Microsoft Corporation, Redmond, USA).

Data collection

Permission was obtained and a schedule was prepared in consultation with the school authorities regarding the sequence of interventions to be carried out on the premises of 6 selected schools. Oral hygiene practices, like the frequency of tooth brushing, brushing aids, the oral rinsing habit, and sugar exposure, were recorded through a self-designed questionnaire at baseline.

Restoration of the teeth in the SDF group

In the children from group 1 (SDF), the identified carious lesion in the affected tooth was efficiently isolated, and petroleum jelly was applied to protect the adjacent teeth and mucosa. Silver diamine fluoride (Fagamin® Silver Diamine Fluoride 38%; Milestone Healthcare, Raipur, India) was applied directly to the lesion with a micro-brush and left for 2 min, and then the tooth was cleaned using a dampened cotton pellet. The treated tooth was restored with GIC (Fuji® IX; GC Corporation, Tokyo, Japan). Excess was removed using a cement carver after checking for high points by asking the patient to bite gently. The parents were instructed to prohibit the child from eating or drinking for 30 min after the application.^{12,17,18}

Restoration of the teeth in the ART group

In the children from group 2 (ART), carious dentin was removed with spoon excavators of different sizes – small (Ash 153/154), medium (Ash 131/132) and large (Ash 127/128), depending on the cavity size. Caries was removed using circular scooping motions and the cavity was widened with a dental hatchet (Ash 10-6-12). The prepared cavity was conditioned for 10–15 s, using a dentin conditioner that comprised 10% polyacrylic acid. Glass ionomer cement (Fuji IX; GC Corporation) was mixed according to the manufacturer's instructions and the cavity was filled in increments, using the blunt end of the carver (Ash 6). The finger-press technique was used to contour the excessive restorative material along the slopes of the cusps. The operating area was kept moisture-free for 2 min and the bite was checked for occlusion. Post-restorative instructions were to avoid the intake of food and drinks for a minimum of 1 h.^{3,5,7,19}

Follow-up examination

The follow-up examinations were carried out at 3-, 6- and 9-month intervals by one of the researchers (DS). The evaluation was done using the community periodontal index (CPI)-probe (WHO-probe) and a mouth mirror. The treated lesions were classified according to the evaluation scores which were modified from those used by Francis et al.²⁰: score 1 – the restoration intact, covering all pits and fissures; score 2 – the restoration partially lost, the tooth is sound (no active/soft caries); score 3 – the restoration partially lost, the tooth is carious (active/soft caries); score 4 – the restoration completely lost, the tooth is sound; and score 5 – the restoration completely lost, the tooth is carious. The tooth was considered sound if its surface felt hard and shiny on gentle probing, and carious in case of soft, demineralized surface. The teeth with the restorations lost and with caries were restored using the conventional treatment in hospital. Due to the loss to follow-up, only 180 restorations were available after 9 months (Fig. 1).

Statistical analysis

All the collected data was recorded and entered into a Microsoft Excel sheet. The descriptive and inferential analyses were done with the use of the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA), by one of the researchers (IM), who was blinded to the treatment options and the evaluation scores. Statistical significance was fixed at a p -value <0.05 . The χ^2 test was used to assess differences between the treatment modalities. The Kaplan–Meier survival analysis was performed using the log rank test to check the survival of both kinds of restorations.

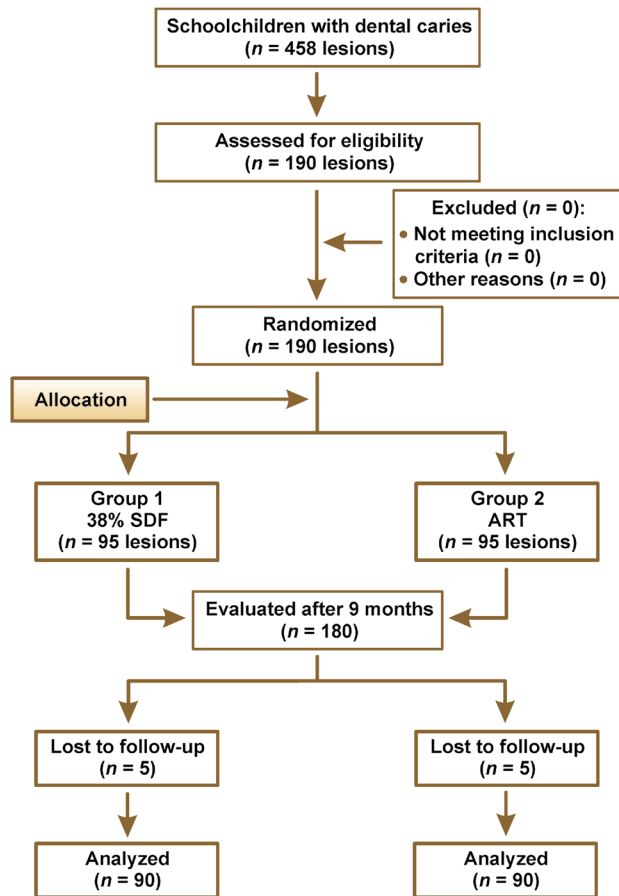


Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) flow diagram of the study

SDF – silver diamine fluoride; ART – atraumatic restorative treatment.

Results

A prospective RCT was conducted to evaluate the effectiveness of 38% SDF with GIC and ART among 6–12-year-old schoolchildren. The demographic variables of the participants are depicted in Table 1. The 2 groups did not differ significantly with regard to these variables at baseline.

In measuring the outcome of the interventions, 58.9% of the participants from group 1 had their restorations intact as compared to 47.8% in group 2, and this was found to be statistically significant ($p = 0.004$) (Table 2). In Table 3, the most common evaluation score among all restorations was ‘an intact restoration covering all pits and fissures’ (53.3%), followed by score 2 (a sound tooth with a partial loss of the restoration). There were no decayed teeth with a complete loss of the restoration (score 5) in group 1. The arrest of dental caries was also better with 38% SDF, as the percentage of carious teeth with the loss of the restoration was 5.6% in comparison with 16.7% in the ART group ($p = 0.025$). The survival analysis using the log rank test showed 38% SDF to be better in comparison with ART (Table 4, Fig. 2).

Discussion

Untreated carious lesions and the underutilization of oral healthcare services are important community health problems in developing and underdeveloped countries. Children are often afraid of anesthetic procedures, long treatment duration and the use of numerous instruments. Hence, treatment involving minimal intervention that is less technique-sensitive is preferred over the conventional dental caries treatment procedures. Many minimally invasive techniques, like ART, which includes the excavation of carious tissue with hand instruments, followed by restoration, have been used for treating dental caries in children and adults. On the other hand, recently, SDF has gained attention because of its caries-arresting and remineralizing effectiveness that is achieved without the excavation of soft carious tissue.²¹ A study conducted by Clemens et al. suggested that SDF was the preferred method for arresting active caries lesions in primary teeth among 2–5-year-old children.¹² Similar findings were also reported in studies by Chu and Lo⁹ and Mei et al.,²² where the application of 38% SDF was better at arresting caries in comparison with fluoride varnish. Yee et al. found SDF to be an effective caries-arresting agent in 3–9-year-old children in Nepal after a follow-up of 12 months.¹¹ Another study by Llodra et al. compared the effectiveness of 38% SDF with ART in the permanent teeth of 6-year-old children; the authors found that SDF reduced caries incidence by 79%.¹⁰ Since the application of SDF is simple and does not require training, it can be implemented by primary healthcare workers in areas of low dental accessibility.

Table 1. Sociodemographic characteristics and oral hygiene practices of the study participants at baseline

Variable	Total N = 190	Group 1 38% SDF n = 95	Group 2 ART n = 95	p-value	
Age [years] M ± SD	9.3 ± 1.3	9.4 ± 1.4	9.3 ± 1.3	0.974	
Gender	male	51.7	45.6	57.8	0.068
	female	58.3	54.4	42.2	
Tooth brushing frequency	once daily	85.6	85.6	85.6	0.584
	twice daily	14.4	14.4	14.4	
Brushing aids	toothbrush	81.7	82.2	81.1	0.500
	other	18.3	17.8	18.9	
Oral rinsing habit	yes	93.3	94.4	92.2	0.398
	no	6.7	5.6	7.8	
Sugar exposure per day	≤5	77.8	83.3	72.2	0.089
	10	16.7	14.4	18.9	
	≥15	5.5	2.3	8.9	

Unless marked otherwise, data presented as relative frequency (%). M – mean; SD – standard deviation.

Table 2. Evaluation scores in the intervention groups after 9 months

Group	Evaluation scores					Total	p-value (χ ² test)
	score 1	score 2	score 3	score 4	score 5		
Group 1 38% SDF	53 (58.9)	19 (21.1)	5 (5.6)	13 (14.4)	0 (0.0)	90	0.004*
Group 2 ART	43 (47.8)	18 (20.0)	15 (16.7)	6 (6.7)	8 (8.9)	90	
Total	96	37	20	19	8	180	–

Data presented as absolute (relative) frequency (n (%)). Scores: score 1 – the restoration intact, covering all pits and fissures; score 2 – the restoration partially lost, the tooth is sound; score 3 – the restoration partially lost, the tooth is carious; score 4 – the restoration completely lost, the tooth is sound; and score 5 – the restoration completely lost, the tooth is carious. * statistically significant.

Table 3. Differences in the evaluation scores between the 2 intervention groups

Evaluation scores	Total	Group 1 38% SDF	Group 2 ART	p-value (χ ² test)
Score 1	96 (53.3)	53 (58.9)	43 (47.8)	0.259
Score 2	37 (20.6)	19 (21.1)	18 (20.0)	0.869
Score 3	20 (11.1)	5 (5.6)	15 (16.7)	0.025*
Score 4	19 (10.6)	13 (14.4)	6 (6.7)	0.108
Score 5	8 (4.4)	0 (0.0)	8 (8.9)	–
Total	180	90	90	–

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

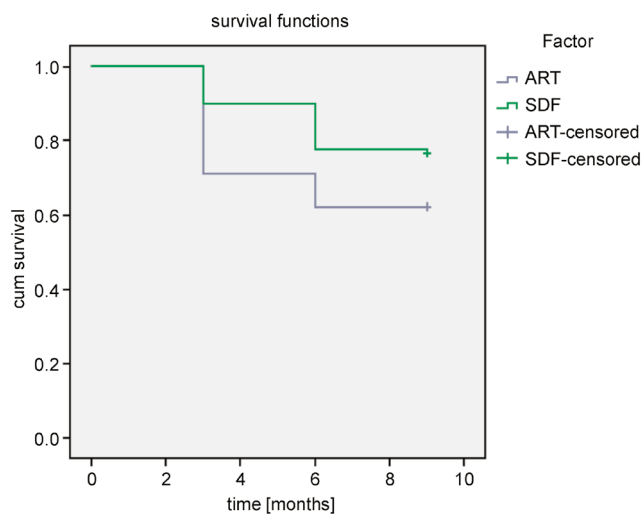


Fig. 2. Survival of the teeth treated with 38% SDF and ART after 3, 6 and 9 months of the intervention cum – cumulative.

The outcome of the restorations was measured and compared between the 2 techniques after 9 months. Caries arrest by SDF was evaluated with scores that estimated the retention or loss of the restorative material, sound or carious soft dentin, along with its color and consistency, which was similar to the studies carried out by Llodra et al.¹⁰ and Fung et al.²³ This evaluation was done using the WHO probe, similar to the studies by Zhi et al.¹ and Dos Santos et al.²⁴ There was no major difference between SDF and ART with respect to score 1, where the restoration was intact, but there was a significant difference between the 2 modalities, with SDF having only 5.6% of the teeth with score 3 as opposed to 16.7% in the case of ART. This was probably due to the remineralization of carious tissue by 38% SDF, which aided in arresting progressive caries. Studies have ascertained that SDF causes remineralization through various probable mechanisms,^{25–28} the first being the occlusion of the dentinal tubules by silver, which demonstrated reduced dye permeability and electric resistance.² Silver and its compounds arrest caries by blocking acid diffusion and sealing the dentinal tubules against the entry of microorganisms. Further growth of microbes is inhibited by the oligodynamic action of silver.²⁵ The other mechanisms could be the reactions between the tooth minerals and SDF. The fluoride released from SDF amplifies the resistance of the dentin, which results in a decreased penetration of acids.²⁶ It has been observed that SDF (Ag(NH₃)₂F) reacts with hydroxyapatite (HA) to release calcium fluoride (CaF₂) and silver phosphate (Ag₃PO₄), which reinforces the affected dentin.⁹ This may be the reason why the teeth were sound and no soft carious tissue was present, even after the restoration was partially or completely lost. This finding is similar with the results of Zhi et al., who concluded that

Table 4. Comparison of the survival rates of the teeth treated with 38% silver diamine fluoride (SDF) and atraumatic restorative treatment (ART) after 9 months of the intervention

Factor	Total	Number of events	Survival of the teeth	SE	95% CI	p-value
SDF	90	21	69 (76.7)	0.210	7.623–8.444	0.023*
ART	90	34	56 (62.2)	0.283	6.446–7.554	
Overall	180	55	125 (69.4)	0.180	7.163–7.870	

Data presented as n (%). SE – standard error; CI – confidence interval; * statistically significant.

the application of an SDF solution or high fluoride-releasing glass ionomer resulted in arresting active dentin caries.¹ Caries arrest by SDF has been reported by various studies, including those by Chu and Lo,⁹ Llodra et al.,¹⁰ Clemens et al.,¹² Fung et al.,²³ and Yamaga et al.²⁹

Silver diamine fluoride showed better retention of the restoration (58.9%) than ART (47.8%) with respect to score 1. The lesser retention in the case of ART is probably the result of the technique-sensitive manual excavation of caries as opposed to the brush application of SDF. As mentioned by Gupta et al., SDF reacts with the tooth surface to produce silver phosphate, which then reacts with RMGIC to form hydroxyapatite and fluorapatite, which may also contribute to increased bond strength.³⁰

The most common side effect of SDF is the staining of the teeth and oral mucosa due to improper isolation. This staining is the result of the oxidation of silver and it affects only the damaged portion of the tooth; healthy enamel is not affected.¹² However, there was no instances of oral mucosal staining in our study.

In the present study, ART scored higher in comparison with SDF with respect to scores 3 and 5, which represented teeth that were carious even after restoration. This could be explained by the fact that caries arrest due to the fluoride release from GIC ceased once the restoration was lost, and hence the remineralizing effectiveness of GIC was also reduced. Other causes could be seepage at the margins of restorations, secondary caries and improper isolation.³¹ The loss of the restoration could be due to the lack of a proper retentive form, as the cavity preparation in ART is done solely with the use of hand instruments. Also, the fact that ART is a technique-sensitive procedure cannot be overlooked.

Our study found that caries arrest was better in group 1, which was reflected by 58.9% of the teeth recording score 1. Similar findings were also reported in studies conducted by Zhi et al.,¹ Llodra et al.,¹⁰ Fung et al.,²³ and Vollú et al.³² The analysis also showed that the survival of the SDF restorations was higher than in the case of ART, and this was similar to the findings of Raskin et al.³³ This may be a consequence of the remineralizing effect of SDF without requiring a proper cavity form, technical skills and or the use of complex armamentarium. Due to the abovementioned advantages of SDF, it can be used with little or no training by primary healthcare workers in areas with the shortage of dental manpower.

Limitations

The current study was conducted over a period of 9 months; a longer follow-up would give a clearer idea about the retention of restorations. Certain outcomes associated with the use of SDF, such as the staining of the teeth and mucosa, was not assessed, as that was not part of the primary objective of the study. The occurrence of secondary caries could not be evaluated, as radiographs

were not available in the field setting where the study was undertaken. Further studies could explore the cost effectiveness of SDF as opposed to ART.

Conclusions

Silver diamine fluoride was found to be an easy, effective and well-received minimally invasive approach for treating dental caries among schoolchildren. The treatment of the teeth with SDF does not necessitate the presence of a well-equipped dental set-up or the use of costly instruments. Hence, SDF can be used effectively in developing countries as a caries-arresting agent in community-based programs.

Trial registration

The trial was registered at Clinical Trials Registry – India (www.ctri.nic.in) (REF/2020/06/034629).

Ethics approval and consent to participate

The study was approved by the Ethical Review Board of the Institute of Medical Sciences and SUM Hospital at Siksha ‘O’ Anusandhan Deemed to be University, Bhubaneswar, India (ref. No. DMR/IMS.SH/SOA/180316). Informed written consent was obtained from the parents before clinical examinations and verbal assent from the children at the time of the procedure.

Data availability

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

Consent for publication

Not applicable.

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Evaluation of the effect of pediatric drugs and an oral rinse on primary teeth discoloration

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

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Abstract

Background. Changes in the color of the teeth are a common dental finding associated with clinical and esthetic problems. Especially, the discoloration of primary teeth can cause parental concern and have a negative effect on social interactions between preschool children.

Objectives. The aim of the study was to evaluate the effect of pediatric drugs and an oral rinse on the discoloration of primary teeth.

Material and methods. Similar to medication intake recommendations, 7 primary teeth in each group were immersed for 1 min in one of 11 different solutions at 8-hour intervals for 1 week. The color values were obtained using a spectrophotometer (VITA EasyShade®) at baseline and after 1 week. The color change (ΔE^*) values were calculated according to the CIELab system. The statistical analysis was conducted using the one-way analysis of variance (ANOVA) and Tukey's post hoc tests at a p -value < 0.05 .

Results. When comparing values L^* , a^* and b^* at baseline and day 7, a statistically significant difference was found in the a^* value for the teeth immersed in pseudoephedrine ($p = 0.012$). There were also statistically significant differences with regard to color change at day 7 (ΔE^*) between the pseudoephedrine and chlorhexidine as well as pseudoephedrine and control groups ($p = 0.034$ and $p = 0.030$, respectively).

Conclusions. The ΔE^* value for pseudoephedrine was 3.7 after 1 week, indicating that it may have the potential to cause significant tooth discoloration when used for a long period. Clinicians and children's families should be aware of the fact that some pediatric drugs can cause tooth discoloration.

Keywords: pediatric, discoloration, drugs, primary teeth

Cite as

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Introduction

Tooth discoloration is a common dental finding associated with clinical and esthetic problems.¹ The discoloration of primary teeth can cause parental concern and have a negative effect on social interactions between preschool children.² Pigment accumulation can be caused by exogenous (foods, beverages and metals) and endogenous factors (systemic diseases or pulp necrosis).³

Cationic antiseptics, such as chlorhexidine and cetylpyridinium chloride, and other mouthwashes may cause staining after prolonged exposure.⁴ However, most evidence suggests that in an anionic diet,^{5,6} the activated chromogens are a possible cause of staining.⁷ Some systemic drugs, e.g., minocycline, doxycycline, amox-clav (amoxicillin + clavulanic acid), and linezolid,^{8,9} have also been shown to cause either extrinsic or intrinsic staining. The pH value, color and viscosity of the drug can play a role in the discoloration of the teeth.^{8,9}

In the treatment of pediatric patients, liquid pharmaceutical dosage forms, such as syrups, solutions and suspensions, are preferred.¹⁰ Although these liquid medications generally have a short duration of usage, they can be used chronically in diseases like respiratory allergies, asthma, attention deficit disorder, and epilepsy. Repetitive use for recurrent diseases, such as tonsillitis, otitis media, sinusitis, and allergic rhinitis, can also lead to significant exposure.¹¹ The use of medications is particularly high in children under the age of 2.¹² An increased exposure to such medications leads to tooth discoloration.¹³

Despite the presence of various types of measurements assessing tooth discoloration, the wavelength dispersive spectrophotometry is considered to be the most accurate method to evaluate tooth staining.² The spectrophotometer is used to measure the tooth color by evaluating 3 color components independent of the surface being studied.¹⁴ These measurements are preferred, as they are more reproducible and objective than visual assessment.¹⁵

Although many studies discuss the influence of medications on the discoloration of permanent teeth, few studies refer to primary teeth.^{2,9} This is an important subject, since primary teeth are different from permanent teeth in some respects – they have pores of a higher volume, thinner and more irregular calcific enamel, and lower mineral content – which makes them more prone to discoloration.¹⁶

The objective of the present study was to evaluate the effect of drugs containing sweeteners and colorants on primary teeth. This study aimed to raise awareness about the use of prescription drugs that cause tooth discoloration, as determined by their chemical and physical properties, and the duration of usage.

Null hypothesis (H0): Different types of pediatric drugs and oral rinses will not cause the discoloration of primary teeth.

Material and methods

This study was approved by the Scientific Research Ethics Committee at the Faculty of Medicine of Karadeniz Technical University, Trabzon, Turkey (No. 2017/86). The approval from the ethics committee was in compliance with the Declaration of Helsinki.

Teeth used

A total of 84 healthy, non-carious primary incisor teeth that had been recently extracted due to pre-shedding mobility were used in this study. Informed consent for the use of the extracted teeth was obtained from the parents of each patient. Teeth with developmental defects, enamel restorations, extrinsic/intrinsic stains, or white spot lesions were excluded from the study. Upon the collection of the teeth, they were placed in 0.5% chloramine T solution (Norateks Chemical Industry, Istanbul, Turkey) for 2 months. Prior to use, the teeth were cleaned with pumice water (Imipomza®; Imicryl, Konya, Turkey) by using a polishing brush (FlexiBrush®; Cosmedent, Chicago, USA) with a low-speed handpiece (KaVo Dental, Biberach an der Riss, Germany).

pH measurement

The medicines and solutions to be tested were prepared. The pH of these solutions was measured using a pH meter (HI 2211 pH/ORP meter; Hanna Instruments, Woonsocket, USA) before starting the experiment (Table 1).

Immersion cycles

The teeth were randomly divided into 12 groups ($n = 7$), with one group determined as the control group, and immersed in an electrolyte solution (sodium chloride (0.4 g/L), potassium chloride (0.4 g/L), calcium chloride-H₂O (0.795 g/L), sodium dihydrogen phosphate-H₂O (0.69 g/L), sodium sulfate-9 H₂O (0.005 g/L), and 1,000 mL distilled water). The color of each tooth was measured at baseline and 1 week after immersion. Before the measurements, each tooth was washed with distilled water for 30 s, cleaned with a soft toothbrush (Colgate® Kids Barbie toothbrush; Colgate Palmolive, Mumbai, India) and gently air-dried.

Each test group was immersed for 1 min in a different pediatric drug solution and 1 oral rinse 3 times a day at 8-hour intervals for 1 week. After each immersion cycle, the teeth were washed with distilled water and kept in an electrolyte solution until the next cycle. The drug solutions and the electrolyte solution were refreshed every day.

Table 1. Characteristics of the products used in the study

Therapeutic class	General name	Brand name	Pharmaceutical form	Manufacturer	pH	Colors	Potential colorants
Antibiotics	amoxicillin + clavulanic acid	Augmentin®	dry powder to prepare oral suspension	GlaxoSmithKline, London, UK	5.70	white-yellow	strawberry cream flavor
	clarithromycin	Macrol®			5.15	white-yellow	titanium dioxide
Anti-epileptics	levetiracetam	Keppra®	syrup	GlaxoSmithKline	5.83	transparent	grape flavor
Multivitamins	ferroglycol sulfate + vit. B1 + vit. B6 + riboflavin + sodium phosphate	Ferro Sanol® B	syrup	Adeka, Samsun, Turkey	2.86	yellow	ferrous(II) glycine sulphate complex, orange essence, pear essence
	vit. A + vit. D + vit. E + vit. K + iodine + selenium + omega-3	Nutrigen® Omega-3			4.56	pink	strawberry flavor
Analgesics	paracetamol	Calpol® 6 Plus	syrup	GlaxoSmithKline	6.62	orange	orange flavor, Sunset Yellow FCF
	ibuprofen	Dolven®			4.38	orange	Quinoline Yellow, Sunset Yellow FCF
Anxiolytics	hydroxyzine HCl	Atarax®	syrup	UCB Pharma, Brussels, Belgium	2.84	transparent	hazelnut flavor
Bronchodilators	salbutamol	Ventolin®	syrup	GlaxoSmithKline	3.58	transparent	orange flavor
Sympathomimetics	pseudoephedrine HCl	Sudafed®	syrup	GlaxoSmithKline	2.76	red	raspberry essence, Ponceau 4R
Oral rinse	benzidamine HCl + chlorhexidine gluconate	Kloroben®	liquid	Drogsan, Ankara, Turkey	5.59	light blue	mint essence, lemon essence, Quinoline Yellow, Patent Blue V
Electrolyte solution	sodium chloride, potassium chloride, calcium chloride-H ₂ O, sodium dihydrogen phosphate-H ₂ O, sodium sulfate-9 H ₂ O, and distilled water	–	–	–	7.00	transparent	–

Shade assessment

The color values for each sample were recorded by means of a spectrophotometer (VITA EasyShade®; Ivoclar Vivadent, Ellwangen, Germany), using the Commission internationale de l'éclairage (CIE) L*a*b* system, where the L* value measures the lightness or brightness of an object, a* is the measure of redness (positive) or greenness (negative), and b* is the measure of yellowness (positive) or blueness (negative).¹⁷

During the measurements, the teeth were placed in a viewing booth, which was completely covered with neutral gray cardboard and had a 5,500 K fluorescent lamp that imitated daylight at the top. The color measurements were taken at the center of the vestibular surface of the tooth. Three measurements were taken for each tooth and the average of these measurements was recorded. By using the L*, a* and b* numerical values, the color change (ΔE^*) values were calculated with the following formula (Equation 1).

$$\Delta E^*(L^*a^*b^*) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

where:

ΔL^* – difference between the L* values measured at different times on the same tooth;

Δa^* – difference between the a* values measured at different times on the same tooth; and

Δb^* – difference between the b* values measured at different times on the same tooth.

Statistical analysis

The statistical analysis was performed using the SPSS Statistics for Windows software, v. 17.0 (SPSS Inc., Chicago, USA). The normality of the data was tested using the Shapiro–Wilk test. The one-way analysis of variance (ANOVA) and Tukey's post hoc tests were used for comparing the ΔE^* values among different groups. The paired sample *t* tests were used for comparing the L*, a* and b* values at baseline and day 7. Both kinds of tests were conducted with a significance level set at $p < 0.05$.

Results

Shade assessment analysis

The one-way ANOVA revealed that there were statistically significant differences with regard to color change at day 7 (ΔE^*_7) between the pseudoephedrine and chlorhexidine as well as pseudoephedrine and control groups ($p = 0.034$ and $p = 0.03$, respectively) (Table 2).

Considering changes in the L^* , a^* and b^* values between baseline and day 7 (ΔL^* , Δa^* and Δb^*) in each group, a statistically significant difference was found in the a^* value for the sample immersed in the pseudoephedrine drug solution ($p = 0.012$). There were no statistically significant differences in the L^* , a^* and b^* values for the samples immersed in the other drug solutions or for the control group ($p > 0.05$) (Table 3). The highest and lowest ΔE^*_7 values were found for pseudoephedrine (3.7) and chlorhexidine (1.6), respectively (Table 2).

pH measurement analysis

The pH values of the evaluated drugs ranged from 2.76 (pseudoephedrine) to 6.62 (paracetamol). Clarithromycin, Ferro Sanol[®] B, Nutrigen[®] Omega-3, ibuprofen, hydroxyzine, salbutamol, and pseudoephedrine had pH below the critical value (5.5), while amoxicillin + clavulanic

acid, levetiracetam, paracetamol, and benzidamine HCl + chlorhexidine gluconate had the pH values above 5.5 (Table 1).

Discussion

The null hypothesis (H_0) was rejected. No significant differences were found in the L^* , a^* and b^* values for the teeth immersed in various drug solutions except for the teeth immersed in pseudoephedrine. These results can be related to many factors, such as the structure of the teeth, their susceptibility to coloring, the pH value, color and viscosity of the drug, and oral rinses.¹⁸

The systemic or local usage of pediatric drugs can cause erosion, caries as well as tooth discoloration related to such factors as the pH value, acidic component and viscosity of the drug.

Table 2. Comparison of the color change at day 7 (ΔE^*_7) values for the pediatric drugs and the oral rinse used in the study

Drug name	ΔE^*_7
Amoxicillin + clavulanic acid	2.2 ± 1.0
Clarithromycin	1.8 ± 0.5
Chlorhexidine gluconate	1.6 ± 0.6[#]
Vit. A + vit. D + vit. E + vit. K + iodine + selenium + omega-3	2.8 ± 1.9
Ibuprofen	2.8 ± 2.3
Hydroxyzine HCl	2.6 ± 1.9
Paracetamol	2.3 ± 1.4
Levetiracetam	2.1 ± 1.3
Pseudoephedrine HCl	3.7 ± 2.1
Salbutamol	2.2 ± 0.9
Ferroglycol sulfate + vit. B1 + vit. B6 + riboflavin + sodium phosphate	2.8 ± 1.4
Electrolyte solution	1.6 ± 1.1^{##}

Data presented as mean ± standard deviation ($M \pm SD$). Statistically significant differences were found between the pseudoephedrine and chlorhexidine[#] as well as pseudoephedrine and electrolyte solution (control)^{##} groups ($p = 0.034$ and $p = 0.030$, respectively).

Table 3. CIELab color parameters for the pediatric drugs and the oral rinse used in the study at baseline and after 7 days of treatment cycles

Drug name	L^*_0	a^*_0	b^*_0	L^*_7	a^*_7	b^*_7
Amoxicillin + clavulanic acid	91.9 ± 2.2	-1.2 ± 1.4	19.4 ± 3.2	90.5 ± 5.0	-1.4 ± 1.6	20.3 ± 6.4
Clarithromycin	91.2 ± 2.2	-1.5 ± 0.9	19.3 ± 4.0	91.7 ± 2.8	-1.3 ± 1.3	19.7 ± 3.9
Chlorhexidine gluconate	90.4 ± 4.4	-1.5 ± 0.9	18.3 ± 5.7	90.2 ± 4.1	-1.7 ± 1.0	18.1 ± 5.7
Vit. A + vit. D + vit. E + vit. K + iodine + selenium + omega-3	88.3 ± 4.1	-0.9 ± 0.9	19.1 ± 3.8	88.1 ± 3.7	-1.1 ± 0.9	18.6 ± 4.5
Ibuprofen	91.8 ± 5.0	-1.6 ± 0.7	19.2 ± 1.9	92.5 ± 3.8	-1.5 ± 0.8	18.4 ± 2.0
Hydroxyzine HCl	92.7 ± 2.9	-1.3 ± 1.3	19.0 ± 2.2	93.5 ± 2.5	-1.8 ± 0.7	17.9 ± 1.7
Paracetamol	90.7 ± 3.8	-0.8 ± 1.5	21.3 ± 4.9	91.9 ± 3.5	-0.7 ± 1.5	21.9 ± 5.3
Levetiracetam	91.6 ± 1.7	-0.7 ± 0.9	20.8 ± 3.5	91.6 ± 2.2	-1.3 ± 0.9	19.6 ± 3.2
Pseudoephedrine HCl	91.6 ± 2.9	-0.9 ± 0.9	19.9 ± 3.5	89.6 ± 6.1	6.6 ± 6.2	19.1 ± 3.9
Salbutamol	92.8 ± 2.9	-1.1 ± 0.7	20.0 ± 2.5	93.0 ± 2.1	-1.5 ± 0.9	19.8 ± 2.9
Ferroglycol sulfate + vit. B1 + vit. B6 + riboflavin + sodium phosphate	88.4 ± 6.4	0.0 ± 2.3	21.6 ± 9.0	88.3 ± 4.9	-0.4 ± 1.4	22.5 ± 6.7
Electrolyte solution	92.9 ± 1.7	-0.9 ± 0.7	20.9 ± 2.6	92.5 ± 1.7	-0.9 ± 0.7	20.2 ± 2.2

Data presented as $M \pm SD$. A statistically significant difference was found in the a^* value in the pseudoephedrine group ($p = 0.012$)

These problems may affect primary dentition, which is important for a child's psychology, nutrition, speaking, esthetics, and permanent dentition.^{19–21} Generally, researchers have studied the discoloration of dental materials and permanent dentition by medications^{6–8,18,22}; however, there is limited knowledge about the effects on primary dentition.^{2,9} The commonly used pediatric drugs may influence the texture of primary teeth and result in their discoloration.

Saliva is known to be a protective factor against the development of erosion and caries in the teeth. The protein-based pellicle covers the surface of the tooth and prevents contact between the tooth surface and acids that can cause demineralization.^{23,24} Investigators have reported that some drugs, iron salts and certain antimicrobials can cause the extrinsic discoloration of the teeth, which is reduced by brushing over the pellicle.^{21,22} Moreira et al. reported the discoloration of bovine teeth caused by different mouthwashes.²⁵ The alcohol-containing types of mouthwash can produce more significant changes as compared to other oral rinses. The alcohol content and a low pH value are associated with the demineralization of the teeth. The lack of significant differences in the group of oral rinses containing 0.15% benzydamine HCl and 0.12% chlorhexidine gluconate is thought to be related to not using any foods or drinks that would cause the pellicle formation; chlorhexidine causes discoloration through the plaque formed on the tooth surface.^{19,20} In this study, the use of an electrolyte solution rather than artificial saliva may have prevented the pellicle formation on the observed teeth. This can be considered a limitation of the study, and can be associated with color changes that may not be clinically relevant.

Tüzüner et al. reported that different types of drugs were associated with the potential risk of discoloring dental materials.¹⁸ Ayaz et al. observed that anti-asthmatic drugs caused the discoloration of dental materials.¹⁷ In the present study, similar to previous research,^{2,8,19,20} all drug groups caused color alterations, but only the pseudoephedrine group reached statistical significance. When comparing primary and permanent dentition, primary teeth have pores of a higher volume, thinner and more irregular calcific enamel, lower mineral content, and a different chemical composition of the pellicle.¹⁶ Thus, the structure of the teeth, the enamel and dentin thickness, the color of dentin, and/or microcracks may be related to discoloration²⁶ and affect the color changes observed on the tooth surface.²⁷ Due to these natural differences, in the current study, discoloration was found to be statistically significant only in the pseudoephedrine group. Additionally, each tooth can display a range of colors from the gingival margin to the incisal edge,²⁸ and thus may show different degrees of discoloration when compared with another tooth despite the same oral conditions. To eliminate this effect, the color values were measured at the same surface area in each tooth in our study.

Pani et al. reported that the different iron syrups evaluated in terms of their effects on primary teeth, including syrups of a low iron content, caused less discoloration than other drug groups.² Although the reasons for the discoloration caused by iron drugs are not clear, the most plausible theory is linked to the pellicle. Among the etiological factors, dietary components, beverages and medications are indicated in all populations, while in children, chromogenic bacteria are also considered.²⁸ Besides, the lesser color change due to the iron-containing medication, Ferro Sanol B, observed in the present study can be related to the shorter time of its usage. Prior to the color analysis of each sample, the teeth were cleaned with the help of a soft toothbrush. Therefore, it is thought that the initial discoloration may have been decreased.²⁹

As mentioned above, investigators have reported that drugs of lower pH or a higher sugar content may demineralize the teeth, and cause erosion and caries.^{16,21,30} Thus, low-pH drugs can cause changes in the tooth surface properties, like roughness,^{11,20} and enhance the adhesion of color pigments.²² It is known that rough surfaces retain stains better than smooth surfaces.²² In the present study, the pH values of each drug were measured and the lowest pH value was noted for pseudoephedrine. Therefore, the significant discoloration observed in the pseudoephedrine group can be related to the drug having lower pH as compared to other drug groups, which affected the tooth surface and increased the adhesion of colorants, in accordance with the colorant absorption theory.²²

In the present study, even though there was no difference in the L^* , a^* and b^* values at the end of day 7 ($p > 0.05$), the color of the teeth in the pseudoephedrine group turned reddish, as observed with an increase in only the a^* value ($p = 0.012$; $p < 0.05$). This red color may be related to the color of pigments and their absorption by the tooth surface. Ponceau 4R (Food Red 7, Acid Red 18, E124) is a water-soluble, sulfonated azo dye widely used in the food industry to provide the red color.³¹ The raspberry essence, and particularly Ponceau 4R, are the ingredients responsible for the red color of pseudoephedrine. It is thought that Ponceau 4R, which has an absorbance at 510 nm wavelength, causes apparent discoloration that does not go away with brushing due to the percolation of pseudoephedrine into the dentinal tubules.

The ΔE^* value indicates whether there is a color change between 2 time periods.²⁵ This refers to perceptibility and acceptability thresholds for color differences in dental matching. Clinical color matching can be graded according to the ΔE^* values. The ΔE^* value represents the numerical distance between the coordinates L^* , a^* and b^* and the relative color variations an observer reports for the materials.²² According to Dancy et al., when the ΔE^* value is 0, it indicates a perfect color difference, 0.5–1.5 means very good, 1–2 means good, 2–3.5 refers to a clinically detectable change, and more than 3.5 to an unacceptable change in color.³²

According to the findings of our study, the sample immersed in pseudoephedrine exceeded the limit of a clinically acceptable color change ($\Delta E^* > 3.5$) (Fig. 1). This can be explained by the fact that colorants in this drug had different polarities. Components with a higher polarity are eluted first, while those with a lower polarity are eluted later. Although there are yellow colorants in both coffee and tea, discoloration by coffee has been reported to be more profound due to the absorption and adsorption of its polar colorants into/onto the surface of materials.³³ Similarly in our study, although some discoloration could be removed by brushing, the polar colorants of pseudoephedrine were absorbed into and adsorbed onto the surface of teeth to a greater extent.

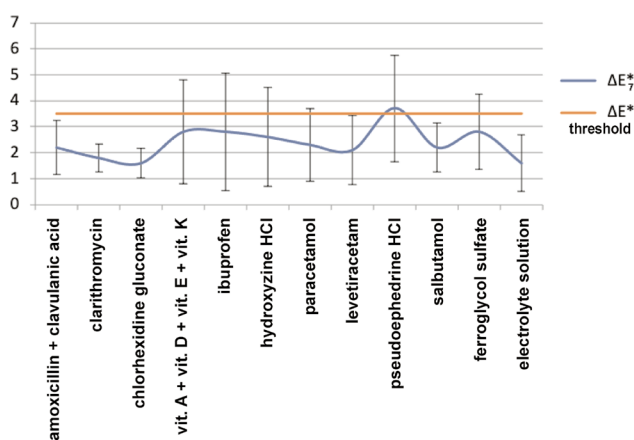


Fig. 1. Comparison of the color change at day 7 (ΔE^*_7) values for the samples immersed in various drug solutions with reference to the ΔE^* threshold of 3.5 (clinical color matching)

In our study, the teeth were kept in 0.5% chloramine T solution. This is the recommended solution for infection control in research projects, as it does not affect the organic phase of dentin. However, it may influence the bonding strength to dentin with a long-term use.³⁴ This should be kept in mind, as it may have negatively affected the in vitro conditions of our study.

No regular brushing of the teeth was performed in the present study. However, after each immersion cycle, the samples were placed in an electrolyte solution, following immersion in distilled water. This procedure was meant to imitate the drinking of water or rinsing with water after the intake of each drug. In future studies, brushing may be recommended to determine how much true staining will occur. In this study, brushing was conducted only before the measurements – at baseline and day 7. In the latter case, it was performed to check the tenacity of the stains and whether they were reversible. In this study, the drugs were evaluated for a duration of 1 week. However, some drugs are used for longer periods of time, which may have a greater effect on the discoloration of primary teeth. In vivo studies are recommended to further evaluate the effect of various drugs on tooth discoloration.

Conclusions

Within the limitations of the present study, it can be reported that, contrary to H0, pseudoephedrine caused the discoloration of primary teeth. It produced a statistically significant difference in the a^* value after 1 week of use as compared to baseline. This situation should be taken into consideration by the physicians prescribing this drug.

Ethics approval and consent to participate

This study was approved by the Scientific Research Ethics Committee at the Faculty of Medicine of Karadeniz Technical University, Trabzon, Turkey (No. 2017/86). The approval from the ethics committee was in compliance with the Declaration of Helsinki. Informed consent was obtained from the parents of each participant.

Data availability

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

Consent for publication

Not applicable.

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Relationship between the *TAS2R38* and *TAS1R2* polymorphisms and the dental status in obese children

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Abstract

Background. The sweet taste and bitter taste genes are thought to have an influence on obesity and caries, which are chronic diseases.

Objectives. The aim of the study was to investigate the effects of the polymorphisms of *TAS2R38* (the bitter taste gene) and *TAS1R2* (the sweet taste gene), which are the most important members of the taste gene family, on the dental status of obese and normal-weight children.

Material and methods. The study included 78 healthy children and 100 children diagnosed with obesity (5–16 years old). The anthropometric measurements and dental status of the children were evaluated. The decayed, missing and filled permanent/primary teeth (DMFT/dmft) index was determined using the standard methods recommended by the World Health Organization (WHO). Blood samples were collected from all subjects and were analyzed via the polymerase chain reaction (PCR) test, with the use of specific primers for the genetic analysis. Five single-nucleotide polymorphisms (SNPs) of the *TAS2R38* and *TAS1R2* genes were investigated. The truncated Poisson and truncated negative binomial modeling approaches were used with regard to the data.

Results. The DMFT/dmft scores were low in obese children and high in children who did not sense the bitter taste (non-tasters). While obese non-taster children had increased DMFT/dmft scores, normal-weight non-taster children had decreased DMFT/dmft scores.

Conclusions. The alanine, valine and isoleucine (AVI) as well as proline, alanine and valine (PAV) haplotypes of the *TAS2R38* gene are associated with the DMFT/dmft index and obesity. This study showed that the DMFT/dmft scores were decreased in obese children. According to the haplotype analysis of the *TAS2R38* gene, the DMFT/dmft scores were increased in non-tasters. When differentiating obese non-tasters and control non-tasters, DMFT/dmft increased in obese non-taster patients, while it decreased in control non-taster patients.

Keywords: genetic, childhood obesity, tooth decay, oral health, *TAS2R38* polymorphism

Introduction

Caries and obesity are multifactorial diseases having a genetic, epigenetic and dietetic background.¹ Like tooth decay, obesity has become an almost global epidemic among children.² Consuming food with a high carbohydrate (CH) content is closely associated with obesity. Therefore, the CH-weighted eating habits represent a common problem related to tooth decay and obesity.³ There is an apparent correlation between caries and CH consumption as a severe risk factor for obesity.⁴

Carbohydrate consumption, which actively contributes to caries and obesity, may be affected by the genetic code determining our taste perception.⁵ The sense of taste helps humans predict whether the food they consume is beneficial or harmful. The taste has developed as a guide to identify the nutrients needed by the body.⁶ Humans can be categorized into 2 groups with regard to their reactions to the bitter taste – tasters and non-tasters. The most critical component responsible for the bitter taste is phenylthiocarbamide (PTC).⁷ Individuals sensitive to PTC are also susceptible to more than 10,000 other tastes, which is not observed in individuals who are not sensitive to PTC.⁸ The primary gene responsible for PTC sensitivity is *TAS2R38*, located on chromosome 7.⁹ The polymorphisms of the *TAS2R38* bitter taste gene may lead to differences in how the taste of food is sensed. Therefore, any polymorphism of this gene may affect nutritional habits, which play significant roles in the development of tooth decay and obesity.¹⁰ Studies are being carried out to discover the effect of *TAS2R38* on the body mass index (BMI).¹¹ In a recent study, it was shown that the daily energy intake of the ‘non-taster’ group was higher in pre-adolescents as compared to the ‘taster’ group, which enjoys the PTC/6-n-propylthiouracil (PROP) taste.¹² It indicates that *TAS2R38* can be associated with increased BMI and obesity risk in children.¹³ Although the *TAS2R38* polymorphisms have been studied in different populations, there is a lack of data with regard to the Turkish population.

Tooth decay, which is caused by many factors, is one of the most common chronic diseases of childhood. Tooth decay occurs as a result of the complex interaction of many environmental and genetic factors.¹⁴ The relationship between *TAS2R38*, obesity and caries has been stated in the literature.¹⁵ Studies on *TAS2R38* show that low taste sensitivity is associated with a greater risk of caries as compared to high taste sensitivity, which suggests that *TAS2R38* may be associated with caries.¹⁶ The *TAS1R* and *TAS2R* gene families encoding taste receptors are involved in the molecular mechanism of taste perception. The *TAS1R* gene family includes the genes encoding receptors that allow the perception of sweet taste; it comprises 3 taste receptor genes on chromosome 1 – *TAS1R1*, *TAS1R2* and *TAS1R3*.¹⁷ The perception of the sweet taste is mediated by the heterodimeric G protein-coupled receptors encoded by *TAS1R2* and *TAS1R3*.¹⁷ A significant relationship between *TAS1R2*

and caries was observed in a recent study.¹⁸ Yet another study, conducted on twins, showed that genetic differences between individuals influenced taste perception and nutritional habits.¹⁹ However, the research on the topic is still scarce in the literature.

The aim of the present study was to investigate the effects of obesity and the polymorphisms of the *TAS2R38* and *TAS1R2* genes, the major members of the taste gene family, on tooth decay in children through evaluating the caries levels in obese and non-taster children. To this end, 2 hypotheses were tested:

- hypothesis I: There is no difference in the incidence of tooth decay between children with obesity and those of normal weight; and
- hypothesis II: There is no difference in the incidence of tooth decay based on the occurrence of the *TAS2R38* bitter taste and *TAS1R2* sweet taste gene polymorphisms.

Material and methods

This cross-sectional, observational study was approved by the Ethics Committee of the Health Sciences Institute at Atatürk University, Erzurum, Turkey (No. B.30.2.ATA.0A10000-617), and was supported by the Scientific Research Projects Commission at Atatürk University (project No. 2012/66 and 2012/375). The study was carried out in collaboration with the Department of Pediatric Endocrinology and the Department of Medical Genetics of the Faculty of Medicine, and the Department of Pediatric Dentistry of the Faculty of Dentistry at Atatürk University. All participants and their parents were informed about the study aims and procedures before any interventions were made, and written consent was obtained from all parents to confirm their approval for treatment. A patient follow-up form was completed for each participant, and the oral and general health status, weight and height measurements, regular medication use, previous dental treatment, and the eating habits over the previous 3 days were recorded. A total of 100 patients aged 5–16 years who had been diagnosed with obesity after referral to the Department of Pediatric Endocrinology were transferred to the pedodontist liable for the assessment of their oral care (MK). The blood samples (2 mL) obtained from the patients as part of routine examinations were collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes and transferred to the Department of Medical Genetics for the genetic analysis. The control group comprised 78 healthy children aged 6–16 years who had been referred to the Department of Pediatric Dentistry and had no systemic disorders. The same procedures were followed for the control group.

Weight measurements were performed using an electronic scale with a ± 100 g sensitivity range when the participants were wearing lightweight clothes. Each participant stood on the scale with both feet placed at the center

of the platform and the value presented on the electronic screen was recorded in kg.

A steel tape measure was used to determine the height of the children while standing upright with their bare feet parallel and adjacent to each other, and with their shoulders and hips touching the wall. Height measurements were recorded in cm. The growth curves described for Turkish children were used to assess BMI,²⁰ and the participants with BMI corresponding to the 95th percentile or above of age-specific limits were considered obese. The decayed, missing and filled teeth of the participants were identified, and the data was recorded for permanent and primary teeth based on the DMFT/dmft scale, respectively.

DNA isolation and SNP genotyping

Whole blood samples were taken from the children for DNA isolation. The blood samples were added by means of venipuncture into Vacutainer[®] EDTA tubes (BD, Franklin Lakes, USA) and used for manual DNA isolation, utilizing the QIAamp[®] DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A single-nucleotide polymorphism (SNP) identification assay (LightSNiP; TIB Molbiol, Berlin, Germany) employing the simple-probe fluorescence melting curve analysis (FMCA) was used to detect the *TAS2R38* and *TAS1R2* polymorphisms. The Rotor-Gene Q (Qiagen) was used in the polymerase chain reaction (PCR) and high-resolution FMCA procedures. A 20-microliter mixture was prepared for each SNP; it included 1 μ L of LightSNiP reagent mix (TIB Molbiol), 2 μ L of LightCycler[®] FastStart DNA Master HybProbe (Roche Diagnostics, Mannheim, Germany), 1.6 μ L of MgCl₂ (25 mM), and a volume of 50 ng DNA and H₂O. The PCR program included the initial denaturation at 95°C for 10 min, followed by 45 cycles of 10-second denaturation at 95°C, 10-second annealing at 60°C and elongation at 72°C for 15 s. The high-resolution FMCA program included cycles at 95°C for 30 s, at 40°C for 120 s and a ramp to 75°C at a transition rate of 1.5°C/s, with the continuous collection of fluorescence. The amplification and melting curves were generated using the Rotor-Gene Q software. As a result of the analysis, the allelic discrimination of the SNPs was achieved.

Statistical analysis

The obtained data was analyzed with the use of the IBM SPSS Statistics for Windows software, v. 21.0 (IBM Corp., Armonk, USA). Descriptive statistics were calculated for each variable and expressed as mean \pm standard deviation ($M \pm SD$). First, the Kolmogorov–Smirnov and Levene tests were applied to determine whether the data showed normal distribution and the homogeneity of variances. The χ^2 test was used to evaluate gender distribution for children in the obese and control groups. The one-way analysis of variance (ANOVA) was used to evaluate

differences with regard to age, DMFT/dmft and body mass index percentile (BMIP). The abovementioned software package was used to analyze the data, and the results were evaluated at an alpha level of 0.05 within a 95% confidence interval (CI). The Poisson and negative binomial models were applied to the data, using the R program (<https://www.r-project.org>). The DMFT/dmft score was defined as the dependent variable, and obesity and the absence of the bitter taste were defined as the independent variables. Caries was modeled with the obesity and non-taster dummy variables by using the truncated Poisson and truncated negative binomial modeling approaches.

The obesity and non-taster dummy variables were created to denote obesity. Obese and non-taster children were coded as 1, and normal-weight children (control) and tasters were coded as 0. Obese non-tasters and control non-tasters were included in the model and labeled as the obese_non-taster and control_non-taster dummy variables (obese non-tasters and control non-tasters were coded as 1, and obese tasters and control tasters were coded as 0). Considering that the highest number of decayed teeth would be 28 in the study, given the maximum age of 16 years in the study population, both models were truncated in the right truncate. The Wald test was used to assess whether the model coefficients were statistically different from zero.²¹

Results

There were no statistically significant differences in gender distribution ($p = 0.881$) or the mean age ($p = 0.312$ and $p = 0.165$ for boys and girls, respectively) between the obese and control groups. No statistical difference was found between the groups in terms of DMFT/dmft. The mean BMIP value was significantly higher in the obese group than in the control group ($p < 0.001$) (Table 1).

Table 1. Gender distribution, age, the number of decayed, missing and filled permanent/primary teeth (DMFT/dmft), and the body mass index percentile (BMIP) values for obese and control patients

Characteristic		Obese group	Control group	<i>p</i> -value
Gender distribution <i>n</i> (%)	total	100 (56.18)	78 (43.82)	0.881
	M	46 (56.79)	35 (43.21)	
	F	54 (55.67)	43 (44.33)	
Age [years] <i>M</i> \pm <i>SD</i>	total	11.80 \pm 2.53	10.89 \pm 3.50	0.050
	M	11.45 \pm 2.34	10.71 \pm 3.67	0.312
	F	12.16 \pm 2.74	11.05 \pm 3.55	0.165
DMFT/dmft <i>M</i> \pm <i>SD</i>		2.80 \pm 3.13	3.29 \pm 3.93	0.352
BMIP <i>M</i> \pm <i>SD</i>		96.27 \pm 3.79	66.22 \pm 14.36	0.000*

M – male; F – female; *M* – mean; *SD* – standard deviation; *statistically significant.

TAS1R2 genotypes and the DMFT/dmft score

When the genotypes of *rs4920566* SNP were evaluated, the highest DMFT/dmft score was found in the AG heterozygous control group. When the genotypes of *rs9701796* SNP were evaluated, the highest DMFT/dmft value was found in the CC homozygous obese group. However, the difference between the genotypes were not statistically significant (Table 2).

TAS2R38 genotypes and the DMFT/dmft score

When the genotypes of *rs713598* SNP were evaluated, the highest DMFT/dmft score was found in the homozygous control group. However, the difference between the genotypes was not statistically significant. When the genotypes of *rs1726866* SNP and *rs10246939* SNP were evaluated, the highest DMFT/dmft scores were observed in the homozygous control group, but the differences between the genotypes were not statistically significant (Table 3).

TAS2R38 haplotypes and the DMFT/dmft score

When the *TAS2R38* haplotypes were evaluated, no statistical differences were found between the control and obese groups. The highest DMFT/dmft score was found in the CC homozygous genotype in all 3 SNPs of the *TAS2R38* gene (Table 3).

Haplotype frequency

A total of 3 different SNPs of the *TAS2R38* gene were evaluated and the linkage disequilibrium (LD) test was

Table 2. Distribution of the DMFT/dmft scores in the *TAS1R2* genotypes

<i>TAS1R2</i> SNP	Genotype	<i>n</i>	DMFT/dmft score		<i>p</i> -value
			<i>M</i> ± <i>SD</i>	min–max	
<i>rs4920566</i>	AA c	17	3.88 ±3.05	0–10	0.294
	AG c	30	4.10 ±4.80	0–15	
	GG c	31	2.67 ±2.70	0–10	
	AA o	43	2.30 ±2.51	0–8	
	AG o	23	3.09 ±4.69	0–20	
	GG o	34	2.82 ±3.08	0–11	
<i>rs9701796</i>	CC c	2	3.00 ±0.70	2–4	0.354
	CG c	17	2.94 ±3.28	0–10	
	GG c	59	3.04 ±3.92	0–15	
	CC o	3	6.00 ±0.00	6–6	
	CG o	30	3.13 ±4.29	0–20	
	GG o	67	2.39 ±2.74	0–11	

SNP – single-nucleotide polymorphism; min – minimum; max – maximum; c – control group; o – obese group.

Table 3. Distribution of the DMFT/dmft scores in the *TAS2R38* genotypes

<i>TAS2R38</i> SNP	Genotype	<i>n</i>	DMFT/dmft score		<i>p</i> -value
			<i>M</i> ± <i>SD</i>	min–max	
<i>rs713598</i>	CC c	24	4.21 ±3.83	0–20	0.222
	CG c	40	2.98 ±3.54	0–8	
	GG c	14	3.64 ±4.14	0–10	
	CC o	44	3.19 ±4.09	0–15	
	CG o	31	2.39 ±2.45	0–14	
	GG o	25	1.88 ±2.35	0–15	
<i>rs1726866</i>	CC c	20	4.75 ±3.85	0–11	0.270
	CT c	37	2.89 ±3.16	0–15	
	TT c	21	3.29 ±4.39	0–20	
	CC o	27	2.89 ±2.75	0–8	
	CT o	44	2.70 ±3.01	0–11	
	TT o	29	2.38 ±4.04	0–20	
<i>rs10246939</i>	CC c	20	4.45 ±4.05	0–11	0.346
	CT c	37	3.05 ±3.10	0–15	
	TT c	21	3.29 ±4.39	0–20	
	CC o	47	2.32 ±2.94	0–11	
	CT o	23	3.26 ±2.80	0–8	
	TT o	30	2.73 ±4.11	0–20	

performed (Fig. 1) to determine the haplotypes of the respondent children (Table 4). Partial tasters were included in the taster group.

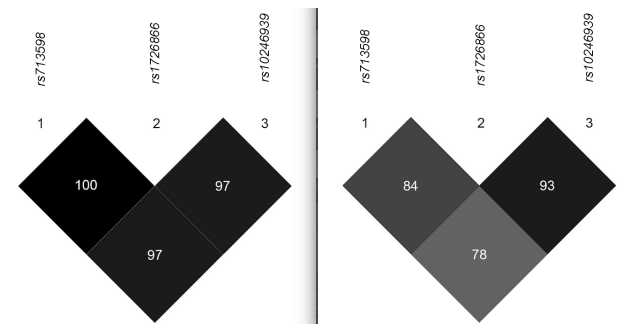


Fig. 1. Linkage disequilibrium (LD) plot for 3 single-nucleotide polymorphisms (SNPs) of *TAS2R38* – *rs713598*, *rs1726866* and *rs10246939*. Numbers indicate the rounded *D'* and *r*² values, while shading indicates the exact *D'* and *r*² values in regard to the distribution of haplotypes in SNPs

Table 4. Haplotype frequency distribution

Haplotype	Obese group	Control group
CCC	98.96 (49.5)	68.00 (43.6)
GCC	4.00 (2.0)	11.00 (7.1)
GTT	90.96 (45.5)	77.00 (49.4)
CCT	2.04 (1.0)	0.00 (0.0)
GTC	4.04 (2.0)	0.00 (0.0)

Data presented as absolute (relative) percentage (*n* (%)). *N* = 200 for the obese group and *N* = 156 for the control group. Frequency <0.03 was ignored in the analysis.

Obesity, non-taster and the DMFT/dmft score

Among the studied SNPs, the *rs713598* (C-G), *rs1726866* (C-T) and *rs10246939* (C-T) base changes were taken into consideration.^{13,21} Children with the haplotype containing a combination of proline, alanine and valine (PAV) constituted the group highly sensitive to the bitter taste. In contrast, children with the haplotype containing the combination of alanine, valine and isoleucine (AVI) constituted the group with the inability to sense bitterness. Children partially sensitive to the bitter taste (i.e., those with haplotypes PAV/AAV, PAV/AVV) were included in the taster haplotype group (PAV) (Table 5). The mean DMFT/dmft scores for the obesity, non-taster, obese_non-taster, and control_non-taster variables are presented in Table 6. The mean DMFT/dmft score for the study population was 3.017, and of these children, 56.2% were obese and 73.5% were non-tasters. Among non-tasters, obese children constituted 42.7% and children with normal weight constituted 29.8% of the population.

Formula 1, which was created to determine the DMFT/dmft index, depending on the obesity and non-taster dummy variants of the child in the Poisson model analysis, is presented below²¹:

$$DMFT = e^{1.109^{***} - 0.172 * OBESITY + 0.121 NONTASTER} \quad (1)$$

where:

e – constant;

*, ***, – statistical significance level of 10% and 1%, respectively.

Table 5. Distribution of the taster and non-taster groups in control and obese children according to the DMFT/dmft index

Haplotype	Group	n	DMFT/dmft score		p-value
			M ±SD	min–max	
PAV	control taster	53	3.60 ±4.63	0–17	0.120
	obese taster	76	1.96 ±2.09	0–13	
AVI	control non-taster	25	3.15 ±3.59	0–14	0.117
	obese non-taster	24	3.07 ±3.36	0–15	

PAV – proline, alanine and valine; AVI – alanine, valine and isoleucine.

Table 6. Mean DMFT/dmft scores for the variants used in the Poisson and negative binomial models

Variable	DMFT/dmft score	
	M ±SD	min–max
DMFT/dmft	3.017 ±3.507	0–20
Obesity	0.562 ±0.498	0–20
Non-taster	0.735 ±0.448	0–20
Obese_non-taster	0.427 ±0.496	0–20
Control_non-taster	0.298 ±0.459	0–20

A minus sign in front of the coefficient indicates a decrease in DMFT/dmft, while a plus sign indicates an increase.

Obesity, obese non-taster, control non-taster and the DMFT/dmft score

The covariates adjusted according to obesity and the absence of the bitter taste data while constructing the Poisson and negative binomial models for the determination of the DMFT/dmft index are presented in Table 7. Before interpreting the analysis results, the likelihood ratio (LR) test was applied to the 2 competitor models. It was observed that the truncated negative binomial model was more compatible with the data.

Formula 2, which was created to determine the DMFT/dmft index, depending on the obesity, obese_non-taster and control_non-taster variants of the child in the Poisson model analysis, is presented below²¹:

$$DMFT = e^{1.2809^{***} - 0.6088^{***} OBESITY + 0.4482^{**} OBESNONTASTER - 0.1332 CONTROLNONTASTER} \quad (2)$$

where:

e – constant;

*, **, *** – statistical significance level of 10%, 5% and 1%, respectively.

A minus sign in front of the coefficient indicates a decrease in DMFT/dmft, while a plus sign indicates an increase.

According to Formula 1, the DMFT/dmft index was lower in obese children and higher in non-tasters. According to Formula 2, obesity reduces the DMFT/dmft index, although the index was increased in obese non-tasters and decreased in normal-weight non-tasters.

Table 7. Coefficients of the Poisson and negative binomial models for the DMFT/dmft scores

Model	Variable	Poisson	Negative binomial
1 st model	constant	1.109 ±0.094***	1.1619 ±0.2161***
	obesity	–0.172 ±0.087*	–0.2334 ±0.2025
	non-taster	0.121 ±0.010	0.1792 ±0.2269
	AIC	1079.11	802.7377
	SBC	1088.65	815.4649
	expected mean DMFT/dmft	3.017	3.020
2 nd model	constant	1.2809 ±0.1054***	1.3864 ±0.2610***
	obesity	–0.6088 ±0.1800***	–0.7053 ±0.3869
	obese_non-taster	0.4482 ±0.1600**	0.4989 ±0.3236
	control_non-taster	–0.1332 ±0.1300	–0.1728 ±0.3180
	AIC	1072.93	803.0060
	SBC	1085.66	818.9095
	expected mean DMFT/dmft	3.017	3.220

AIC – Akaike information criterion; SBC – Schwarz Bayesian criterion; *, **, *** statistical significance level of 10%, 5% and 1%, respectively.

Discussion

It is important to determine individual risk factors for dental caries in order to create a specific treatment procedure for a particular patient.²² Considering the close association between obesity and the eating habits, researchers have identified a link between obesity and tooth decay.²³ Studies to date have yielded controversial results regarding the relationship between obesity and tooth decay, and further studies are required to understand the link better. A previous systematic review demonstrated that 48% of the investigated studies had reported no significant relationship between BMI and tooth decay; in contrast, a positive and a negative relation was found in 35% and 19% of the same studies, respectively.²⁴ Hong et al. reported a positive relationship between BMI and tooth decay in children aged 60–72 months.²⁵ However, this relationship was not significant when age, genetic predisposition and the eating habits were excluded from the analysis.²⁵ In the present study, a comparison of the DMFT/dmft index for obese and normal-weight children was made, revealing a decreased index value in obese children, and thus hypothesis I was rejected. There are several factors that can be considered responsible for the inverse relation noted between DMFT/dmft and obesity. Children with multiple untreated decayed teeth may be undernourished, as they suffer from severe pain. Also, as a cause of infection, restlessness and sleep problems, tooth decay may decrease life quality in these children, negatively affecting their growth and development. Furthermore, most of the control group subjects were referred to our clinic with a complaint of pain and the control group had a higher incidence of tooth decay than expected, which may have affected our findings.

Many studies have indicated that diet, overweight, low weight, caries, and obesity are interrelated in children. Two recent systematic reviews support this assumption.^{26,27} Therefore, it is reasonable to think that dental caries and obesity may be related to sweet taste genetics. The sugar intake and taste preferences have been associated with the *TAS1R2* polymorphisms in humans. While a high sugar intake has been linked with caries and obesity, few studies have examined the genetic background.^{28,29} Various polymorphism studies of the *TAS1R2* gene have revealed a significant relationship between caries and genotypes.^{29,30} In the present study, the risk of caries was higher in normal-weight children; it may have been due not to the lower incidence of caries in obese children, but to the fact that the population already had a high risk of caries.

A meta-analysis study suggested that *TAS2R38* *rs713598* SNP might play an essential role in caries.³¹ However, our study did not show significant results in any of the 3 SNPs, which is similar to a study by Shimomura-Kuroki et al.³² Since the *TAS2R38* gene

haplotype analysis includes the genotype analysis, the results regarding haplotypes are more critical.³³ The physiology of taste has not been fully clarified to date, although the possible effect of the biological mechanism of taste on tooth decay and obesity appears to be worth investigating.³⁴ The results of the present study demonstrate that tooth decay is associated with 3 different polymorphisms of the *TAS2R38* gene, and the taster and non-taster haplotypes formed by these polymorphisms in obese and normal-weight children. Furthermore, the eating habits and the preferred foods, as the major factors affecting obesity and closely related to tooth decay, may be influenced by bitter taste genetics. There have been few studies to date investigating the relationship between taste genes, obesity and tooth decay, and the conducted ones have failed to clarify the relationship fully.^{14,16,35}

The potential links between taste genetics and various disorders have been investigated in several studies.^{36–38} Accordingly, there is a growing interest in the effects of the biological mechanisms involved in taste sensation on the development of tooth decay and obesity.^{32,38,39} Studies investigating the relationship between the *TAS2R38* polymorphisms and tooth decay reported a lower incidence of tooth decay among tasters. Wendel et al. reported that *TAS2R38* was the main gene associated with tooth decay.⁴⁰ A study conducted on infants revealed that *TAS2R38* and *TAS1R2* controlled their nutritional preferences for the weaning foods.⁴¹ On the other hand, a study conducted on adults argued that this effect might change with age, maternal dietary practices and community habits.⁴² Individuals with the PAV haplotype have greater sensitivity to the bitter taste and better gustatory perception. Due to their ability to sense different flavors, their tendency to cariogenic foods may be reduced.³⁶ In the present study, the participants were classified into tasters and non-tasters, based on the haplotype analysis of the *TAS2R38* gene. Three haplotypes of this gene generated by 3 different SNPs were examined (the corresponding amino acids were A49P, V262A and I296V). In the investigated SNPs, the *rs713598* (C-G), *rs1726866* (C-T) and *rs10246939* (C-T) base exchanges were taken into account. According to the present study results, tooth decay decreases in the presence of obesity and increases in the absence of the bitter taste. However, in the subgroup analysis of non-tasters, the DMFT/dmft index was raised in obese non-tasters and lowered in normal-weight, control non-tasters, and thus hypothesis II was rejected. The authors suggest an increased risk of caries in the obese non-taster group. Further research on this topic can be done. More studies may be needed to explain this issue more clearly. Accordingly, the authors are planning to conduct different studies involving the same patient population. For example, the level of taste can be evaluated by applying solutions of bitter taste and the results can be compared with the genetic analysis.

This study has some limitations, such as keeping the sample size low, and not investigating other causes of caries and obesity.

The topic of taste has not been given the attention it deserves in dentistry or other branches dealing with the sensation of taste. The sensation of taste is a field that is not yet fully understood and there are many gaps in the acquired knowledge. There are branches in medicine that deal directly with the 4 senses of sight (eye), hearing (ear), touch (skin), and smell (nose). However, no specialty field exists for the sense of taste (tongue). The sense of taste and its genetics require support from studies, including the present one.

Conclusions

The CC control homozygous genotype showed higher caries rates in all SNPs of the *TAS2R38* gene, although not statistically significantly. Although there were no statistically significant differences in the DMFT/dmft index with regard to *TAS1R2 rs9701796* SNP in both control and obese groups, a high DMFT/dmft score was observed in the CC homozygous genotype. This may suggest that the CC genotype of *TAS2R38* and *TAS1R2* is prone to caries. Advice including dietary recommendations, increased oral hygiene and controlled calorie intake can be given to children with the identified taste gene polymorphisms.

Ethics approval and consent to participate

This cross-sectional, observational study was approved by the Ethics Committee of the Health Sciences Institute at Atatürk University, Erzurum, Turkey (No. B.30.2.ATA.OA10000-617), and was supported by the Scientific Research Projects Commission at Atatürk University (project No. 2012/66 and 2012/375). The participants' parents provided informed written consent prior to any 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.


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Comparative study between using a tissue adhesive (N-BCA & OCA) and surgical sutures in free gingival graft surgery: A randomized controlled clinical trial

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Conflict of interest

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Abstract

Background. The success of periodontal surgery depends on the appropriate coaptation of the incised edges, the elimination of empty spaces and the reduction of the amount of blood coagulum. In free gingival graft (FGG) surgery, the stabilization of the graft, hemostasis and cleansing the site remain a necessity.

Objectives. The aim of the present study was to compare the use of the tissue adhesive N-butyl cyanoacrylate + octyl cyanoacrylate (N-BCA + OCA; Iceberg-glue[®]) and surgical sutures to evaluate the stabilization and fixation of FGG, which is indicated to increase the width of the keratinized attached gingiva.

Material and methods. A total of 24 cases – 12 patients aged 18–45 years with gingival recession and the absence of the keratinized gingiva underwent FGG surgery at 2 parallel sites at the same time. A long piece of FGG was taken from the palate, from the area adjacent to a canine to a second molar, and divided into 2 equal pieces. The bed of the graft was prepared at each side with a 15C blade. One piece was applied to each bed, one graft was sutured using 0/5 nylon sutures and the other one was stabilized using the tissue adhesive.

Results. The data analysis showed that the postoperative pain ended after 3 days at the tissue adhesive site, but it continued up to 4 days at the suture site. The healing index was significantly better after 2 months at the tissue adhesive site. Graft shrinkage was significantly lower after 3 months at the tissue adhesive site, whereas there was no significant difference in graft shrinkage between the 2 methods at 6 months after the surgery.

Conclusions. The Iceberg-glue tissue adhesive proved to be a reliable alternative material to sutures in stabilizing the free gingival graft at the recipient site. It reduced postoperative pain and improved the healing of the recipient site.

Keywords: tissue adhesive, cyanoacrylate, free gingival graft, keratinized attached gingiva

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Introduction

The survival of a free gingival graft (FGG) depends on multiple factors, including the appropriate coaptation of the incised edges, the avoidance of any empty spaces and the reduction of the amount of blood coagulum. In FGG surgery, the stabilization of the graft, stopping the bleeding and cleansing the bed of the graft remain a necessity.¹ Sutures are the most popular technique for the appropriate coaptation of the graft; however, new materials and mechanisms are being studied in order to achieve and maintain the stability of the graft in its bed, and to prevent the invasion of any foreign bodies.^{2,3} Using cyanoacrylate tissue adhesives in FGG surgery, has proved to be easy and effective, and it also causes fewer problems as compared to the use of sutures. In addition, due to their antibacterial properties, tissue adhesives decrease the risk of a toxic effect.^{4,5}

Free gingival grafting, introduced in 1968,⁶ is a routine clinical procedure in oral surgery. The inadequate amount of the attached gingiva presents a big surgical problem. In addition, it does not allow patients to control plaque effectively. The localized gingival inflammation at a specific site is considered as a sign of the insufficient width of the attached gingiva, regardless of the overall satisfactory oral hygiene. The novel clinical and histological research suggests that any width that allows the maintenance of healthy periodontium can be regarded as sufficient.⁷

Free gingival grafting is commonly utilized to increase the width of the attached gingiva.⁸ It is considered as the gold standard in this regard due to its autogenous character, the ease of technique and the possibility of maintaining of the keratinized tissue. In addition, in case of FGG, surgical outcomes can be predicted.⁹

Different amounts of graft shrinkage have been noticed during the healing period.^{10,11} The permanent nutrition of the graft affects graft shrinkage; there must not be any factors that disrupt the continuity of graft nutrition. As stated by Sullivan and Atkins, the thickness of the graft, an atraumatic surgical procedure and the rapid fixation of the graft are essential for preserving graft vessels from any harm and dehydration, thereby minimizing the chances of shrinkage.⁶ Accordingly, the number of sutures should be decreased to minimum, since every suture induces a localized hematoma under the graft, which may increase graft shrinkage. For that reason, the sutureless stabilization techniques might help to minimize or even prevent graft shrinkage; adhesives might be an appropriate method to achieve this target.¹¹

Tissue adhesives were introduced for the first time as an alternative to sutures by Ardis in 1949.¹² The performance of cyanoacrylate tissue adhesives was examined by Coover et al. in 1959; it was chemically analyzed and suggested for application in surgical pro-

cedures.¹³ Tissue adhesives were employed in the early 1960s, but unluckily, the resultant inflammatory reactions were not acceptable. Therefore, many modifications have been made to minimize their unfavorable inflammatory effects. Other problems related to the use of tissue adhesives were the lack of good training and the incorrect use resulting from by the insufficient knowledge in this field.

Several types of tissue adhesives have been developed and improved due to continuous research and technological development, and they have been approved and licensed by the U.S. Food and Drug Administration (FDA).¹⁴

Cyanoacrylates are a type of tissue adhesives that have been developed 50 years ago to repair injuries.¹⁵ In 1974, Binnie and Forrest compared butyl cyanoacrylates and silk sutures in the stabilization of periodontal flaps, and they found lesser edema and swelling as well as a better gingival contour in the butyl cyanoacrylate group.¹⁶

Free gingival graft surgery was done for the first time without the use of any sutures by Hoxter in 1979.¹ He thought that the graft could survive without any sutures only if it had been positioned and fixed at the recipient site correctly. He postulated that it was possible to obtain the intended stability, sterility, and even homeostasis.¹

N-butyl cyanoacrylate + octyl cyanoacrylate (N-BCA + OCA) (Iceberg-glue®; GMI, Barcelona, Spain) is a new combination of tissue adhesives, manufactured by blending 2 different kinds of cyanoacrylate, giving the adhesive better flexibility, while keeping high levels of resistance. Actually, scientific literature illustrates that N-BCA is a cyanoacrylate molecule with the highest tensile strength (34.27 N) and low flexibility.¹⁷ On the other hand, OCA has a lower tensile strength (11.27 N) and at the same time it has better elastic features. The Iceberg-glue adhesive has an excellent tensile strength, in addition to good elasticity due to the presence of OCA. In addition to the abovementioned characteristics, this formula has hemostatic, sealing, antibacterial and adhesive properties. Polymerization starts about 2 s after the application and is completed after 60 s. The temperature generated during the polymerization process is about 45°C,^{18,19} which is lesser than for other kinds of cyanoacrylates.^{20,21} The intraoral use of tissue adhesives is limited to superficial application.²² Cyanoacrylate tissue adhesives keep their adhesive properties even in the presence of humidity.²³

There are not enough studies that have aimed to evaluate wound healing and assess patient-centered outcomes. The aim of this study was to compare patient-centered outcomes, postoperative pain, wound healing, and the shrinkage of FGG between two recipient sites – one fixed with sutures and the other with cyanoacrylate tissue adhesives.

Material and methods

Trial design

This study is a two-arm, split-mouth, randomized controlled clinical trial comparing a tissue adhesive (Iceberg-glue) and surgical sutures with regard to the levels of pain, the modified early-wound healing index (MEHI) and the shrinkage of the graft.

This randomized clinical trial (RCT) was approved by the institutional Ethics Committee at the Faculty of Dental Medicine of the University of Damascus, Syria (No. 2773MS). The protocol was registered in the ISRCTN registry (ISRCTN10301784).

Our study recruited patients who were referred to the Department of Periodontology, Faculty of Dental Medicine, the University of Damascus, between April 2019 and June 2019. The procedure was fully explained to all patients and written informed consent was obtained from them.

Sample size

The G*Power software, v. 3.1.7 (<https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower>), was used to calculate the sample size with an alpha level of 0.05 and a power of 80 (based on previous research²⁴); a sample size of 24 cases was required (12 patients).

Eligibility criteria

All patients who required FGG surgery to increase the amount of the keratinized tissue at 2 parallel sites, i.e., had the attached gingiva lower than 1 mm, were considered as eligible candidates for our study.

Those who were older than 18 years, and had the plaque score and the bleeding score <20% were enrolled. Patients with uncontrolled diabetes or coagulation disorders (with liver disease, on the anticoagulative therapy, etc.), those who took corticosteroids, or had any systemic disorder that would disturb oral and periodontal surgery were excluded. Figure 1 illustrates the process of recruitment.

Allocation concealment

The patients were asked to choose a sealed envelope to decide which site was going to be stabilized with sutures and which one with the tissue adhesive. The envelopes were unsealed just before the surgery. The follow-up measurements were done by another dentist (W.A) who did not have any information regarding our current trial.

Description of the intervention

This RCT was done on 24 cases – 12 patients aged between 18–45 years who had an insufficient amount of the attached gingiva and FGG was recommended.

All patients underwent an initial periodontal therapy that consisted of providing oral hygiene instructions, motivating, scaling, polishing, and root planing if necessary. All patients were examined at baseline and 2 months after the completion of the initial periodontal treatment to determine if there was a necessity to increase the width of the attached gingiva or not. The plaque and bleeding scores in the whole mouth were <20% at the recall visit.

One long piece of the graft was harvested from the palate, from the area between a second molar and a first premolar; then, it was divided into 2 equal parts and the thickness of the graft was standardized between the 2 sides, as the graft thickness is an important factor affecting the shrinkage of the graft.⁶

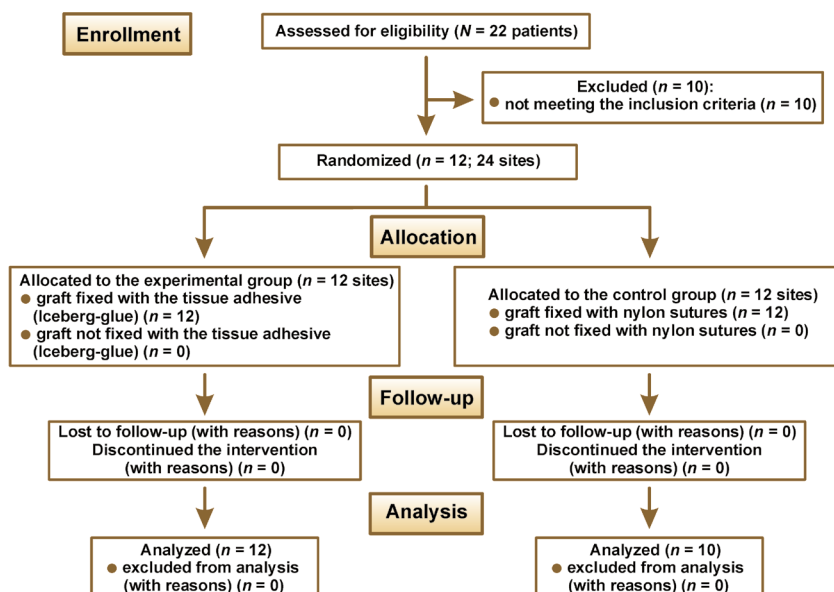


Fig. 1. Flow diagram of the patients' recruitment and follow-up

The graft was positioned in its bed and held pressed for 2 min for initial stability. At one side, the graft was stabilized using 0/5 nylon sutures (interrupted sutures to the papilla and a sling suture were placed to adapt the graft intimately to the root and periosteal bed),²⁵ while at the other side, FGG was stabilized with the tissue adhesive (Fig. 2–4).

The blood supply between FGG and the recipient site is essential for the healing process, as well as atraumatic graft fixation for the maintenance of plasmatic circulation.²⁴

Outcomes and follow-up

The primary outcome was the shrinkage of the graft, which was assessed at 2 months, 3 months and 6 months after the surgery. The length and width of the graft were measured using the University of North Carolina-15 (UNC-15) periodontal probe. The secondary outcomes were the recipient site healing and the recipient site pain.

The recipient site healing was assessed by means of MEHI²⁶ at 1 week, 2 weeks, 1 month, and 2 months after the surgery. The healing progress was evaluated using the Landry, Turnbull and Howley healing index, which classifies healing on the basis of redness, and the presence



Fig. 2. Preoperative view

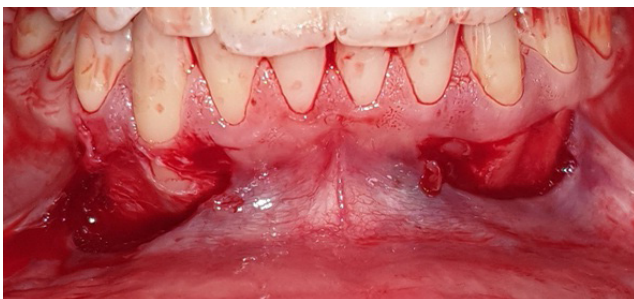


Fig. 3. Initial incision

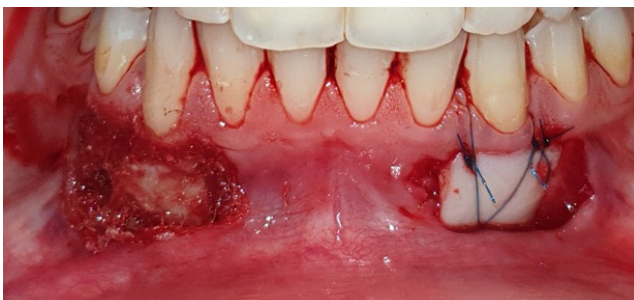


Fig. 4. Fixing the graft

of bleeding, granulation tissue, epithelialization, and supuration, and rates it from score 1 (very poor healing) to 5 (excellent healing) accordingly.²⁷

The recipient site pain was assessed by the patient with the use of a 1–10 visual analog scale (VAS).²⁸ A questionnaire sheet was given to the patients to document the levels of pain from the recipient site before taking any analgesics at 6 h, 12 h, 24 h, 2 days, 3 days, 4 days, 5 days, 6 days, and 7 days after the surgery.

Analgesics were taken only when needed (ibuprofen 500 mg, PRN), a mouth rinse (chlorhexidine 0.12%) was prescribed twice a day for 1 week, and instructions were given to avoid eating any solid or hot food in the 1st week after the surgery. The patients were also instructed to return 1 week after the operation to remove sutures and to bring the questionnaire sheet.

Statistical analysis

The data analysis was carried out by means of the SPSS Statistics for Windows software, v. 17.0 (SPSS Inc., Chicago, USA), using a two-tailed test with alpha set at 0.05. Descriptive statistics were reported as mean and standard deviation ($M \pm SD$) for continuous variables, and as frequency and percentage (n (%)) for categorical variables.

The data was analyzed at baseline and during the postsurgical follow-up with parametric tests, when the variables were distributed normally. To evaluate ordinal variables, non-parametric tests were implemented; the Mann–Whitney U test when the 2 variables were not related, while the Friedman and Wilcoxon tests declared that differences within the same variable were checked across multiple time points. The required assumptions for each of the aforementioned tests were insured before conducting them.

Results

12 patients (24 cases) were recruited from the start and were eligible for analysis. Their mean age was 27.0 ± 8.4 years. The percentage of females was 75% ($n = 9$). The patients' baseline characteristics are shown in Table 1.

Table 1. Baseline characteristics of the groups

Variable	Tissue adhesive group $n = 12$	Suture group $n = 12$	p -value
Gender	male	3 (25)	1.000
	female	9 (75)	
Age [years]	27.0 ± 8.4	27.0 ± 8.4	1.000
Side	right	5 (41.7)	0.414
	left	7 (58.3)	

Data presented as number (percentage) (n (%)) or as mean \pm standard deviation ($M \pm SD$).

Table 2. Graft size [mm²] according to the way of fixation of the free gingival graft (FGG)

Time point	Way of fixation	Number of sites	Graft size [mm]	p-value	Lowest rate	Highest rate
During surgery	tissue adhesive	12	43.25 ±7.03	1.000	32	55
	sutures	12	43.25 ±7.03		32	55
After 2 months	tissue adhesive	12	28.83 ±6.37	0.741	18	40
	sutures	12	28.00 ±5.83		18	40
After 3 months	tissue adhesive	12	25.08 ±5.12	0.043*	18	36
	sutures	12	21.08 ±3.92		12	27
After 6 months	tissue adhesive	12	19.08 ±4.78	0.264	12	28
	sutures	12	16.83 ±4.84		10	24

Data presented as $M \pm SD$. * statistically significant.

Table 3. Pain scores (visual analog scale (VAS)) in both groups

Time point	Tissue adhesive group n = 12	Suture group n = 12	p-value
After 6 h	6.92 ±2.07	5.83 ±1.85	0.126
After 12 h	6.42 ±1.88	5.58 ±1.68	0.214
After 24 h	5.08 ±1.88	4.17 ±1.85	0.258
After 2 days	3.58 ±2.07	2.92 ±1.88	0.347
After 3 days	0.83 ±1.34	1.00 ±1.60	0.890
After 4 days	0.42 ±0.90	0.33 ±1.15	0.350
After 5 days	0	0.08 ±0.29	0.317
After 6 days	0	0	1.000
After 7 days	0	0	1.000

Data presented as $M \pm SD$.

A single calibrated examiner (W.A) did all measurements. There was no significant difference between the study groups at baseline in terms of gender, age and graft site.

The size of the graft reduced significantly within the 2 groups at 2, 3 and 6 months after the surgery ($p < 0.05$). After 2 and 6 months, there were no significant differences between the 2 groups. However, after 3 months, the cyanoacrylate group showed significantly less shrinkage as compared to the other group ($p < 0.05$) (Table 2).

The VAS score at the recipient site decreased from day 1 and reached a nadir at day 3 in the tissue adhesive group, while the same pain nadir level was achieved on day 4 in the suture group. Table 3 and Table 4 depict the pain scores in different groups.

Table 4. Patients' responses in the questionnaires administered at 10 assessment time points postoperatively for the pain scores at the 2 sites

Site	Time point	p-value								
		after 6 h	after 12 h	after 24 h	after 2 days	after 3 days	after 4 days	after 5 days	after 6 days	after 7 days
Tissue adhesive	after 6 h	1.000	–	–	–	–	–	–	–	–
	after 12 h	0.157	1.000	–	–	–	–	–	–	–
	after 24 h	0.034	0.046	1.000	–	–	–	–	–	–
	after 2 days	0.005	0.007	0.025	1.000	–	–	–	–	–
	after 3 days	0.002	0.002	0.002	0.002	1.000	–	–	–	–
	after 4 days	0.002	0.001	0.002	0.002	0.102	1.000	–	–	–
	after 5 days	0.001	0.001	0.001	0.002	0.059	0.317	1.000	–	–
	after 6 days	0.002	0.001	0.002	0.002	0.063	0.317	0.317	1.000	–
Suture	after 6 h	1.000	–	–	–	–	–	–	–	–
	after 12 h	0.083	1.000	–	–	–	–	–	–	–
	after 24 h	0.014	0.083	1.000	–	–	–	–	–	–
	after 2 days	0.002	0.002	0.005	1.000	–	–	–	–	–
	after 3 days	0.002	0.001	0.001	0.002	1.000	–	–	–	–
	after 4 days	0.002	0.001	0.002	0.002	0.317	1.000	–	–	–
	after 5 days	0.002	0.002	0.002	0.003	0.046	0.083	1.000	–	–
	after 6 days	0.002	0.002	0.002	0.003	0.046	0.083	1.000	1.000	–
after 7 days	0.002	0.002	0.002	0.003	0.046	0.083	1.000	1.000	1.000	

Wilcoxon signed-rank test.

As to the healing index, there were no significant differences between the 2 sites after 1 week, 2 weeks, 1 month, and 2 months. However, the healing process was completed 1 month postoperatively in the tissue adhesive group, whereas in the suture group, it lasted up to 2 months (Table 5) (Fig. 5–7).

Table 5. Differences in the healing index between the 4 studied periods postoperatively at the 2 sites

Site	Time point	p-value			
		after 1 week	after 2 weeks	after 1 month	after 2 months
Tissue adhesive	after 1 week	1.000	–	–	–
	after 2 weeks	0.007	1.000	–	–
	after 1 month	0.002	0.002	1.000	–
	after 2 months	0.002	0.001	0.157	1.000
Suture	after 1 week	1.000	–	–	–
	after 2 weeks	0.020	1.000	–	–
	after 1 month	0.003	0.002	1.000	–
	after 2 months	0.002	0.001	0.046	1.000

Wilcoxon signed-rank test.



Fig. 5. Two-week follow-up



Fig. 6. Two-month follow-up



Fig. 7. Six-month follow-up

Discussion

In the present RCT, the use of a tissue adhesive and surgical sutures, two different graft stabilization techniques, were compared in terms of recipient site postoperative graft shrinkage, pain levels and healing index (MEHI). Our main findings were that there was no significant difference in graft shrinkage, but the pain levels were significantly lower and healing was significantly better at the tissue adhesive site.

After 3 months, at the cyanoacrylate side, graft shrinkage was significantly lower than at the other side with surgical sutures ($p < 0.05$); however, there was no significant difference between the 2 sides observed at 2 months and the difference disappeared at 6 months after the surgery. It might be attributed to the fact that there was no or very little trauma caused to the bed of the graft at the 2 sides while operating. Our findings are supported by a study conducted by Barbosa et al., who fixed FGG with ethyl cyanoacrylate in one group and with silk mucoperiosteal sutures in the other.²⁴ The mean graft area measured in square millimeters on day 30, day 45 and day 90 was 60.9 ± 15.1 , 54.5 ± 16.1 and 54.5 ± 16.1 in the cyanoacrylate group and 59.0 ± 2.4 , 57.1 ± 1.3 and 57.1 ± 1.3 in the suture group, respectively. No significant difference was found between the study groups for alterations in the grafted area.²⁴ On the contrary, Paknejad et al. observed a significant difference in graft shrinkage 3 months postoperatively after stabilizing FGG with a tissue adhesive and silk sutures.²⁹ Similarly, Gümüş and Buduneli, who compared the traditional technique, the use of a cyanoacrylate tissue adhesive or microsurgery in terms of graft stabilization, observed that graft shrinkage was significantly lesser at the cyanoacrylate site than at the other two.³⁰ The proposed explanations were that a shorter and atraumatic operation in the cyanoacrylate group along with the soft pressure applied to the graft before it was stabilized in its bed had a significant role in maintaining avascular plas-matic circulation, which eventually results in less shrinkage.

The VAS score indicted no pain at the recipient site 3 days postoperatively in the tissue adhesive group, but the pain lasted for 4 days in the suture group. Since graft stabilization with a tissue adhesive appeared in a considerably shorter time, less inflammation and edema were expected; hence, lower postoperative pain. This might explain why the tissue adhesive sites became pain-free rapidly. Zucchelli et al. found that a longer operative time in the control group may have balanced the secondary intention wound healing experienced by the test group in terms of pain in the postoperative period.³¹ Paknejad et al. found no significant differences in pain levels between the 2 groups.²⁹ The contradiction between our findings and those of Parkenjad et al. might be due to the difference in the chemical composition of the tissue adhesives used.

One of the important issues in FGG surgery is the graft and recipient site healing. We found that the tissue adhesive significantly reduced the time needed for healing the

wound and accelerated wound healing. Approximately, we achieved complete wound healing at the tissue adhesive side after 1 month. On the other hand, a 2-month period was needed for complete wound closure at the suture side. Paknejad et al. found a significant difference between the 2 groups in the healing process after 10 days, though there were no significant differences in the healing process after 20 and 90 days.²⁹ It might be due to the fact that they used an ethyl 2-cyanoacrylate-based tissue adhesive; the component is short-chained and rarely used, as it degrades very rapidly and allows for the subsequent formation of formaldehyde, which is very toxic.³²

Strengths and limitations

Our study was the first RCT to evaluate the new brand of cyanoacrylate-based tissue adhesive for the oral mucosa (Iceberg-glue), comparing it with the classical way of suturing. Since it was difficult to blind the sites from the dental surgeon, we implemented a strict protocol to insure that the outcome analyzers were blinded to the sites, using an objective way of assessment. However, our study has some limitations. For example, the assessment of pain scores and some of the questionnaires were subjective in nature. In addition, due to the limited resources, we were not able to recruit more patients, despite efforts to do so, in order increase our sample size.

Clinical significance

A tissue adhesive could be a reliable alternative material to sutures in stabilizing FGJ at the recipient site. It reduced postoperative pain and improved the healing of the recipient site.

Conclusions

In this study, whether the grafts were fixed with N-butyl cyanoacrylate + octyl cyanoacrylate (N-BCA + OCA) or nylon sutures, differences in graft size were not statistically significant. Also, this study suggests that the N-BCA + OCA tissue adhesive is capable of accelerating and boosting the healing of gingival tissue wounds. Further research with longer follow-up periods on tissue adhesives is needed, as postoperative pain was significantly lower at the tissue adhesive site (it ended after 3 days at the tissue adhesive site and after 4 days at the suture site). Therefore, the use of cyanoacrylate may be a promising alternative for the conventional technique to stabilize free gingival grafts.

Trial registration

The trial was registered at the ISRCTN registry (ISRCTN10301784).

Ethics approval and consent to participate

The study was approved by the institutional Ethics Committee at the Faculty of Dental Medicine of the University of Damascus, Syria (No. 2773MS). Informed written consent was obtained from all the patients.

Data availability


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


Consent for publication

Not applicable.

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Comparison of two optical devices used for artificial tooth color selection

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Abstract

Background. Correct color assessment and the selection of the color of the prosthetic restoration are important aspects of prosthetic treatment, which significantly affect the success of the treatment.

Objectives. The aim of this study was to compare 2 commercial devices used for tooth color selection.

Material and methods. The color of maxillary right central incisors and right canines was assessed in a group 100 patients aged 22–40 years (25.11 ± 3.24 years), using the Easyshade[®] spectrophotometer and the ShadeStar[®] colorimeter. Two visual shade guides were used as references for the tests – VITA VITAPAN[®] Classical and VITA 3D-Master. The 2 instruments and the 2 visual shade guides were assessed in terms of agreement in tooth color selection.

Results. There were statistically significant differences between the 2 instruments in terms of agreement in tooth color selection as well as between the 2 shade guides. The VITA VITAPAN Classical shade guide was shown to be more accurate in tooth color selection than VITA 3D-Master. There was agreement between the Easyshade spectrophotometer and ShadeStar colorimeter measurements for incisors in 49% of cases with the VITA VITAPAN Classical shade guide and in 22% of cases with VITA 3D-Master. In the comparative analysis of the Easyshade and ShadeStar devices with regard to the measurements performed on canines there was 52% agreement for the VITA VITAPAN Classical shade guide and 32% agreement for VITA 3D-Master.

Conclusions. The VITA VITAPAN Classical system demonstrated superior agreement in shade selection as compared to the VITA 3D-Master system. A low degree of agreement between the optical devices used in the selection of the color of artificial teeth may indicate optical differences between devices from different manufacturers.

Keywords: tooth color, shade selection, shade guides, spectrophotometer

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Introduction

The dynamic development of dental disciplines observed in recent years has allowed dentists to meet the esthetic expectations of patients in the field of restorative dentistry.¹ The color of the teeth and their shape are the crucial features affecting their appearance.² Correct color assessment and the selection of the color of prosthetic restorations are the most important aspects of prosthetic treatment contributing to patient satisfaction.³ Generally, tooth color is assessed visually, using the color shade guide provided by the manufacturer of dental materials for tooth reconstruction.⁴ The visual method is subjective, as many factors impact color perception,⁵ for example lighting conditions, gingiva color and background colors.⁶ The effects of gender, anxiety, depression, and the use of medicaments should also be taken into consideration.⁷ For these reasons, objective methods are desirable for appropriate tooth color selection. Nowadays, an increasing number of dental offices use optical equipment and software to assess tooth color. Such aids include digital cameras, colorimeters and spectrophotometers.^{8,9} Controlled studies have shown the advantage of using such systems over the traditional visual method.¹⁰ In order to perform prosthetic work, dentists collaborate with various technicians, who may also have an influence on tooth color selection. The use of an optical instrument for assessing the color of natural teeth often gives better results than the visual method.¹⁰ As mentioned above, there are several commercial devices available for assessing color in the context of choosing the right shade of artificial teeth for prosthetic treatment.¹¹ Digital cameras are the simplest instruments used to assess tooth color. Colorimeters consist of a light source, usually a xenon lamp, photodiodes, a detector, and a converter. They use filters that simulate the color response of the human eye.¹² Spectrophotometers are the most accurate and useful of these instruments, but they are also the most expensive.⁹ They measure the amount of light reflected from an object with 1–25-nanometer bandwidths in the visible spectrum.¹³ The 2 main types of spectrophotometers are: spot measurement devices (SMDs) with a small, approx. 3–5-millimeter spot; and complete tooth measurement devices (CMDs) with a large measuring window covering the entire tooth or more than 1 tooth. Those with a limited field may be disadvantageous due to the greater loss of the reflected light, since the path of the reflected light can run outside the sensor window. Both SMDs and CMDs lose some of the incident light due to the absorption of the light beam inside the tooth; the beam passing through the tooth is beyond the range of the sensor.¹⁴ The large-scale use of these instruments is dependent on their availability, affordability, ease of use, and reliability.

The null hypothesis tested was that there is no difference in the chosen tooth color between the 2 color-matching devices. The aim of the present study was to verify whether the use of the optical equipment provided

by different manufacturers for tooth color selection will give reproducible and acceptable results.

Material and methods

Study population and ethical considerations

A total of 100 participants (22 males and 78 females) were recruited to this study. The age of the patients ranged from 22 to 40 years (25.11 ± 3.24 years). Each participant was provided with a full explanation of the study aim and the procedures to be followed. Informed written consent was obtained from the participants before they were enrolled for the study. The study was approved by the Ethics Committee of Poznan University of Medical Sciences, Poland (No. 459/14).

Inclusion and exclusion criteria

The inclusion and exclusion criteria are presented in Table 1. The inclusion criteria required a vital tooth, and maxillary right central incisors and right canines with no cracks, fillings and/or discolorations. The exclusion criteria were as follows: a non-vital tooth; and a tooth with discolorations, fillings, and after root canal treatment or whitening procedures.

Data collection

The color of maxillary right central incisors and right canines was assessed in a group of 100 patients with the use of the Easyshade[®] V spectrophotometer (VITA Zahnfabrik, Bad Säckingen, Germany) and the ShadeStar[®] colorimeter (DeguDent, Hanau, Germany) by the same dental specialist in prosthodontics. Two shade guides were used for the tests – VITA VITAPAN[®] Classical and VITA 3D-Master (VITA Zahnfabrik). Differences in tooth color

Table 1. Inclusion and exclusion criteria

Criteria	Details
Inclusion criteria	a vital tooth
	maxillary right central incisors and right canines
	no cracks
	no fillings
Exclusion criteria	no discoloration
	a non-vital tooth
	a tooth with discolorations
	a tooth with fillings
	a tooth after root canal treatment
	a tooth after whitening procedures

selection with the use of the abovementioned devices were assessed.

The subject teeth were cleaned with gauze, and the device was properly cleaned and disinfected before use. Protective coating was applied to the tip of the probe to avoid damage to the optical system and to prevent the tip from slipping off the tooth being examined. After activating the Easyshade spectrophotometer and allowing the lamp to reach the operating temperature, an automatic calibration process was carried out. The tip of the probe was placed in the spectrophotometer calibration block at an angle of 90°. After proper calibration, the operating mode of the device was selected for the measurement of a single tooth. The tip of the probe was placed perpendicular and close to the tooth surface, 2 mm below the gingival margin and at least 2 mm above the incisal edge of the tooth, to prevent incorrect measurements. The measurement lasted about 2 s. Care was taken not to move the measuring tip during the color test. The probe was pulled away from the tooth after a beep from the instrument, when the result appeared on the Easyshade monitor. The measurement was made at least 3 times on each of the examined teeth and repeated until 2 identical results appeared in succession before the color was recorded.

The same procedure was carried out using the ShadeStar colorimeter.

Statistical analysis

The statistical analysis was performed with Statistica, v. 12.0 (StatSoft Polska, Kraków, Poland), and the MedCalc® statistical software, v. 19.1.7 (MedCalc Software, Ostend, Belgium; <https://www.medcalc.org>). The values are presented as number and percentage distribution (n (%)). Cohen's kappa coefficient was used to measure differences between the devices. The comparative analyses of the percentage of consistent assessment were performed using the paired difference test. The results were deemed statistically significant at $p < 0.05$.

Table 2. Compatibility of the Easyshade spectrophotometer and ShadeStar colorimeter measurements according to the VITA VITAPAN Classical and VITA 3D-Master shade guides ($N = 100$)

Teeth	VITAPAN Classical (%)	κ	3D-Master (%)	κ	p -value
Incisors	49	0.47	22	0.28	0.0001*
Canines	52	0.50	32	0.38	0.0064*

κ – Cohen's kappa coefficient; * statistically significant.

Table 3. Differences between incisors and canines in terms of compatibility of the Easyshade spectrophotometer and ShadeStar colorimeter measurements according to the VITA VITAPAN Classical and VITA 3D-Master shade guides

Variable	VITAPAN Classical			3D-Master		
	incisors	canines	p -value	incisors	canines	p -value
Agreement in tooth color selection between the Easyshade and ShadeStar devices	49	52	0.3193	22	32	0.1111

Data presented as percentage (%).

Results

The comparative analyses of the compliance of the Easyshade spectrophotometer and ShadeStar colorimeter measurements for VITA VITAPAN Classical and VITA 3D-Master showed that the agreement of the Easyshade spectrophotometer measurements with those performed with the ShadeStar colorimeter for maxillary right central incisors according to the VITA VITAPAN Classical key was 49%, while according to the VITA 3D-Master key, it was 22%. The agreement of the Easyshade spectrophotometer measurements with those made with the ShadeStar colorimeter for maxillary right canines according to the VITA VITAPAN Classical key was 52%, whereas according to the VITA 3D-Master key, the compliance was 32% (Table 2).

The comparative analyses of the compatibility of the Easyshade and ShadeStar devices for maxillary right central incisors showed a statistically significant difference between the VITA VITAPAN Classical and VITA 3D-Master dyes ($p = 0.0001$), and likewise for maxillary right canines ($p = 0.0064$) (Table 2).

Cohen's kappa coefficient showed a moderate agreement for the VITA VITAPAN classical shade guide for incisors ($\kappa = 0.47$) and a moderate agreement for VITA VITAPAN Classical shade guide for canines ($\kappa = 0.50$). The comparative analyses of the compatibility of the Easyshade spectrophotometer and ShadeStar colorimeter measurements according to the VITA 3D-Master shade guide showed a fair agreement for incisors ($\kappa = 0.28$) and a fair agreement for canines ($\kappa = 0.38$) with respect to Cohen's kappa coefficient (Table 2).

The comparative analyses of the compatibility of the Easyshade spectrophotometer and ShadeStar colorimeter measurements according to VITA VITAPAN Classical as well as VITA 3D-Master showed no statistically significant differences between incisors and canines ($p = 0.3193$ and $p = 0.1111$, respectively) (Table 3).

Discussion

Spot measurement devices were selected for this research because of the positive evaluation of the instruments by many authors.^{8,15} Kim-Pusateri et al. studied the reliability and repeatability of tooth color measurements with the use of 4 spectrophotometric devices; the results showed high accuracy in the tooth color measurements made with the Easyshade spectrophotometer.⁸ Llana et al.

compared SMD and CMD, noting the high reproducibility of the results in the selection of the color of artificial teeth as measured by the Easyshade device.¹⁵ The sensor diameter of the Easyshade spectrophotometer is 5 mm, and this device operates with 2 VITA shade guides – VITAPAN Classical and 3D-Master. ShadeStar is a colorimeter with a sensor diameter of 3 mm, which operates with 4 shade guides – VITA VITAPAN Classical, VITA 3D-Master, Ceram[®] X-Mono, and Ceram X-Duo. Due to the compatibility of both devices with the same color shade guides (VITAPAN Classical and 3D-Master), it was decided to subject these to a comparative analysis.

In this study, the consistency of measurements between the Easyshade spectrophotometer and the ShadeStar colorimeter while testing the 100 incisors was 49% for VITAPAN Classical and 22% for 3D-Master. By comparison, in the analysis of the Easyshade and ShadeStar measurements made on canines there was 52% agreement for VITAPAN Classical and 32% agreement for 3D-Master. It indicates that the results are not completely satisfactory. The lower compatibility obtained with the 3D-Master shade guide was probably due to the greater number of color shade tabs. Similar observations were made by Sarafianou et al.¹⁶ They examined the consistency of measurements from 2 spectrophotometric devices – Easyshade and SpectroShade[®]. The authors reported a low degree of compatibility for both spectrophotometers, thus suggesting the lack of compatibility of devices from different manufacturers.¹⁶ Such low compatibility between devices can lead to problems in communication with dental technicians, who may check the color of the prosthetic work with a different device than the one used by the dentist when choosing the color of the teeth.

The repeatability of colorimeter measurements may also be low due to the aging of the filters and the metamorphism of the tested objects.¹⁷ Reports regarding the assessment of the accuracy and repeatability of the color measurements performed with the use of a spectrophotometer are contradictory.^{1,8,16,18,19} In vitro studies indicate greater accuracy of spectrophotometric devices.^{8,19} In a few studies, the accuracy of spectrophotometers under clinical conditions was evaluated.^{20–22} However, conducting in vivo tests is more difficult; for example, failing to keep the measuring tip of the device stationary inside the patient's mouth may lead to lower measurement precision.^{8,12} Weyhrauch et al. claimed that the Easyshade spectrophotometer is characterized by high repeatability of measurements, and therefore specialists can use it independently of each other without affecting the quality of tooth color analysis.²³ Measurements with the use of these devices were also carried out under laboratory conditions. Agreement between 3 spectrophotometric devices – Easyshade, SpectroShade and ShadeVision[®] – was checked in the conditions simulating the clinical situation.¹² The measurements were made in 2 different settings – standardized and traditional, in accordance

with the manufacturer's recommendations. The Easyshade spectrophotometer had the highest repeatability under standardized conditions, corresponding to 100% for VITAPAN Classical and 3D-Master colors. While performing measurements as recommended by the manufacturer, i.e., under conditions that simulate those of the dental office, repeatability decreased to 48.2% for the VITAPAN Classical shade guide and to 55.3% for the 3D-Master shade guide.¹² In our study, measurements with the devices were repeated 3 times for each tooth. In the event of the devices showing a variation in color, the measurement was repeated until 2 identical color results were obtained in succession. This approach improves the precision of devices and increases the likelihood of choosing the most acceptable color.^{19,24} In vitro studies have shown that there is a significant difference between the subjective assessment made by the dentist and instrumental tooth color selection in favor of colorimeters.²⁵ Only a few researchers have shown considerable agreement between the visual method and instrumental methods in assessing tooth color under clinical conditions.^{26,27}

Li and Wang suggest that the accuracy of selection is not guaranteed, either by subjective or objective methods, although better results are obtained when using a colorimeter.²⁸ Our research showed poor agreement in the results obtained with the spectrophotometric and colorimetric devices, and statistically significant differences for incisors and canines when using the VITAPAN Classical and 3D-Master shade guides. Judeh and Al-Wahadni reported that spectrophotometers needed improvement, but the combination of measurements by means of these devices with visual assessment produced favorable results.²⁹ Lasserre et al.³⁰ and Śmielecka and Dorocka-Bobkowska⁵ came to similar conclusions, reporting that the highest agreement between visual and computer-assisted selection was achieved when a lamp was used to optimize lighting conditions during the choice of color by the dentist.

Limitations

A limitation of this study is the loss of some colorimetric data due to the reflection or refraction of the beam of light generated in the direction of the tested object; as the teeth are not perfectly flat and opaque, some of the reflected light is not 'captured' by the instrument sensor. Additionally, in the study, the measurements were made in the central part of the tooth; the tests should be extended to the cervical and incisal zones of the tooth, since it is known the teeth are not of a uniform color.

Conclusions

Within the limitations of this study, we concluded that the VITA VITAPAN Classical system demonstrated

superior agreement in shade selection as compared to the VITA 3D-Master system. A low degree of agreement between the optical devices used in the selection of the color of artificial teeth may indicate optical differences between devices from different manufacturers.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Poznan University of Medical Sciences, Poland (No. 459/14). Informed written consent was obtained from the participants before they were enrolled for the study.


Data availability


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

Consent for publication

Not applicable.

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Influence of chronic hyperhomocysteinemia on the features of bone metabolism in the case of lipopolysaccharide-induced periodontitis

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Abstract

Background. Periodontal disease is the second most common oral health problem after dental caries. This increasing prevalence makes it not only a health problem, but also a social issue. The pathogenesis of periodontal disease is associated with a number of adverse exogenous and endogenous factors, including hyperhomocysteinemia (HHcy).

Objectives. This study aimed to determine the features of bone metabolism in rats with lipopolysaccharide (LPS)-induced periodontitis combined with chronic thiolactone HHcy.

Material and methods. Forty-eight white, non-linear, mature rats were divided into 4 groups: control ($n = 12$); LPS-induced periodontitis ($n = 12$); chronic thiolactone HHcy ($n = 12$); and periodontitis combined with HHcy ($n = 12$). The rats were sacrificed the day after the last LPS injection or the day after the last homocysteine (Hcy) thiolactone administration. Bone metabolism was determined based on the activity of alkaline phosphatase (ALP) and acid phosphatase (AP) in blood serum and periodontal homogenate.

Results. A decrease in ALP activity (by 40.1%; $p = 0.001$) and the mineralization index (MI) (3.5 times; $p < 0.001$) with an increase in AP activity (2.0 times; $p < 0.001$) was observed in the periodontal homogenate of rats with LPS-induced periodontitis. In the case of LPS-induced periodontitis combined with chronic thiolactone HHcy, more pronounced changes in the activity of phosphatases and in MI were established as compared to rats with LPS-induced periodontitis only.

Conclusions. Chronic thiolactone HHcy enhances disturbances in bone metabolism in LPS-induced periodontitis. The osteotoxic effect of HHcy is associated with the activation of osteoclastogenesis and enhanced bone resorption. However, further research is required on the subject.

Keywords: periodontitis, bone metabolism, hyperhomocysteinemia

Introduction

Inflammatory disorders that involve both soft and hard periodontal structures, such as gingivitis and periodontitis, are described as periodontal diseases. Nowadays, periodontal disease is the second most common oral health problem after dental caries,¹ and its increasing prevalence makes it not only a health problem, but also a social issue.² The prevalence of periodontal disease in the European population is above 50%, with 10% suffering from a severe form of the disease. The prevalence of the disease increases with age, reaching 70–85% in patients aged 60–65 years.³ In Ukraine, the prevalence of periodontal disease among the general population is ranging from 92% to 98%.⁴

The early identification of periodontitis can help prevent the early loss of teeth. The treatment of periodontitis revolves around the debridement of the plaque biofilm and calculus which accumulate around the dentition. It is also pivotal to perform debridement and employ plaque control measures around dental restorations and at their interface with periodontal hard and soft tissues in order to prevent the initiation and progression of periodontitis. Additionally, the therapeutic strategies used in the treatment of periodontitis include the administration of antimicrobials, anti-inflammatory agents and antioxidants, systemically and topically.⁵

Several factors, of both exogenous and endogenous origin, are associated with the pathogenesis of periodontal disease. One of such factors is a high level of homocysteine (Hcy) – hyperhomocysteinemia (HHcy).⁶ The first study on the association of Hcy with periodontitis appeared in 2004.⁷ In 2015, Bhardwaj et al. suggested using Hcy as a marker of inflammation in patients with periodontitis.⁸ Similar findings, including an increased level of Hcy in both the blood plasma and saliva of patients with inflammatory and destructive periodontal disease were obtained in other studies.^{9–11}

The possible mechanisms resulting in increased Hcy levels in periodontitis involve the hyperproduction of pro-inflammatory cytokines, including interleukins (IL) (IL-1 β , IL-6 and IL-8) as well as tumor necrosis factor alpha (TNF- α), in periodontal tissues. These mediators initiate an inflammatory cascade that can potentially disrupt methionine and Hcy homeostasis, leading to HHcy.^{8,10}

On the other hand, metabolic dysregulation during periodontitis increases systemic inflammation, which leads to a decrease in the levels of vitamins, in particular B6, B12 and folic acid, which play important roles as cofactors in Hcy metabolism.¹²

There are only a few publications that focus on the course of experimental periodontitis in the case of HHcy. Krivosheeva et al. found that experimental HHcy complicated the course of periodontitis in rats.¹³ Probable mechanisms responsible for the negative effect of HHcy on the course of periodontitis

may be the activation of oxidative stress, which primarily causes endothelial damage and the development of endothelial dysfunction,^{14,15} and the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), which is known to participate in the regulation of the adaptive immune response and stimulate the synthesis of pro-inflammatory cytokines (TNF- α , IL-1 β), inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and leukocyte adhesion molecules, which promote the migration of leukocytes into the vessel wall, thereby increasing the cytotoxicity of leukocytes.¹⁶

It is worth noticing that recent evidence points to the same mechanism with regard to peri-implant tissues. Thus, Guarnieri et al. compared gingival tissue healing at the surgically manipulated periodontal sites and at the sites receiving implants and healing abutments with machined (MS) vs. laser-microtextured (LMS) surfaces, placed with one-stage protocol.¹⁷ The researchers found that both MS and LMS implant sites presented a higher pro-inflammatory state in the early phase after surgery (1–4 weeks).¹⁷

This study aimed to determine the features of bone metabolism in rats with lipopolysaccharide (LPS)-induced periodontitis combined with chronic thiolactone HHcy.

Material and methods

Study design

Inbred white male rats ($n = 48$) were housed in a room with controlled temperature ($22 \pm 2^\circ\text{C}$) and a 12/12 h light/dark cycle at the Laboratory Animal Facility of I. Horbachevsky Ternopil National Medical University, Ukraine, for the duration of the experiment. The animals had unrestricted access to food and water. The experimental procedures including animal treatment fully complied with relevant regulations.¹⁸ The experimental design and protocol were approved by the Bioethics Committee of I. Horbachevsky Ternopil National Medical University (protocol No. 64 of May 17, 2021).

The study animals were randomly placed into one of 4 groups as follows: control (group 1; $n = 12$); animals with model periodontitis (group 2; $n = 12$); animals with chronic thiolactone HHcy (group 3; $n = 12$); and animals with periodontitis combined with HHcy (group 4; $n = 12$). The rats in group 2 received injections of 40 μL (1 mg/mL) of *Escherichia coli* (*E. coli*) LPS (Sigma-Aldrich, St. Louis, USA) into gingival tissues every other day for 2 weeks.⁴ The rats in group 3 were administered Hcy thiolactone intragastrically (100 mg/kg of body weight in 1% solution of starch) once a day for 42 days.¹⁹ In group 4, chronic thiolactone HHcy was induced as described above. From the 29th day after the start of HHcy

induction, the animals were injected with LPS into the gum tissue for 14 days concurrently with the ongoing Hcy thiolactone treatment.

The animals were euthanized with cardiac puncture under sodium thiopental anesthesia on the day following the last LPS injection (groups 2 and 4) or the day after the last Hcy thiolactone administration (group 3). The collected blood and periodontal samples were used for further investigations.

To confirm the development of HHcy in the blood serum of the animals, the total Hcy level was determined by means of a solid-phase enzyme-linked immunosorbent assay (ELISA), using an Axis Shield reagent kit (Axis Shield Diagnostics, Dundee, UK) according to the manufacturer's protocol and a Multiskan™ FC analyzer (Thermo Fisher Scientific, Vantaa, Finland). The Hcy level was expressed in $\mu\text{mol/L}$.

Bone metabolism was determined based on the activity of alkaline phosphatase (ALP) as a marker of osteoblast functioning and acid phosphatase (AP) as a marker of the intensification of osteoclast activity. The activity of phosphatases was expressed in $\mu\text{kat/L}$ (in blood serum) or $\mu\text{kat/kg}$ (in periodontal homogenate). The mineralization index (MI) was determined based on the ratio of ALP to AP.

Statistical analysis

The experimental data was compiled and analyzed using Microsoft Office Excel (v. 2016; Microsoft Corp., Redmond, USA) and Statistica, v. 7 (StatSoft Inc., Tulsa, USA). The Kolmogorov–Smirnov test was used to determine the normality of data distribution. If the values did not conform to normal distribution, the Kruskal–Wallis test was performed to compare 3 or more groups, followed by the Mann–Whitney test with the Bonferroni correction for the pairwise comparisons of the groups. The results were presented as median (*Me*) and interquartile (*IQR*) range. The results were considered statistically significant at the probability level (*p*-value) < 0.05 .

The association between the studied indices was established based on the results of the correlation analysis using Spearman's rank correlation coefficient. The linear

correlation coefficient (*r*) and its probability (*p*-value) were calculated. The association was considered very weak at $r = 0.10$ – 0.30 , weak at $r = 0.31$ – 0.50 , moderate at $r = 0.51$ – 0.70 , strong at $r = 0.71$ – 0.90 , and very strong at $r = 0.91$ – 0.99 . The direction of the association, positive or negative (inverse), was also assessed. The correlation coefficient was regarded as statistically significant at $p < 0.05$.

Results

The results showed that the blood serum Hcy level in rats with only LPS-induced periodontitis increased by 47.4% as compared to control, but this change was not statistically significant ($p = 0.215$). In animals with LPS-induced periodontitis combined with chronic thiolactone HHcy, this index increased 3.8 times as compared to control ($p < 0.001$) and was 2.6 times higher than in the case of LPS-induced periodontitis alone ($p = 0.002$). It should be noted that in animals with isolated chronic thiolactone HHcy, the serum level of Hcy increased 3.4 times as compared to control ($p < 0.001$), but it did not differ significantly from the serum Hcy level observed for the group with LPS-induced periodontitis combined with chronic thiolactone HHcy ($p = 0.999$) (Table 1).

We found that the activity of serum ALP in the case of LPS-induced periodontitis decreased by 22.1% as compared to control, but this change was not statistically significant ($p = 0.091$). In animals with isolated chronic thiolactone HHcy, an increase in this index by 94.6% as compared to control was found ($p = 0.001$). In rats with LPS-induced periodontitis combined with chronic thiolactone HHcy, the activity of serum ALP increased by 22.1% ($p = 0.893$) in relation to controls and was by 56.7% higher than in the case of LPS-induced periodontitis alone ($p = 0.005$) (Table 2).

While analyzing ALP activity in periodontal homogenate, it was found that in rats with LPS-induced periodontitis, this index significantly decreased by 40.1% as compared to control ($p = 0.001$). In animals with isolated chronic thiolactone HHcy, the periodontal ALP activity

Table 1. Level of homocysteine (Hcy) in the blood serum of rats with lipopolysaccharide (LPS)-induced periodontitis without comorbid pathology and combined with chronic thiolactone hyperhomocysteinemia (HHcy)

Parameter	Experimental group				Kruskal–Wallis criterion (H), <i>p</i> -value	<i>p</i> -values
	group 1 (control)	group 2 (periodontitis)	group 3 (HHcy)	group 4 (periodontitis + HHcy)		
Hcy [$\mu\text{mol/L}$]	7.70 (7.40–8.15)	11.35 (10.15–11.65)	26.39 (23.99–29.63)	29.04 (26.45–33.12)	26.41 $p < 0.001^*$	$p_{1-2} = 0.215$ $p_{1-3} < 0.001^*$ $p_{1-4} < 0.001^*$ $p_{2-3} = 0.035^*$ $p_{2-4} = 0.002^*$ $p_{3-4} = 0.999$

Data presented as median (interquartile range) (*Me* (*IQR*)). p_{1-2} , p_{1-3} , p_{1-4} , p_{2-3} , p_{2-4} , p_{3-4} – probability of differences between the particular groups (numbers in lowercase correspond to group numbers); * statistically significant.

Table 2. Indices of bone metabolism in the blood serum and periodontal homogenate of rats with lipopolysaccharide (LPS)-induced periodontitis without comorbid pathology and combined with chronic thiolactone hyperhomocysteinemia (HHcy)

Parameter	Experimental group				Kruskal–Wallis criterion (H), <i>p</i> -value	<i>p</i> -values
	group 1 (control)	group 2 (periodontitis)	group 3 (HHcy)	group 4 (periodontitis + HHcy)		
Blood serum ALP [μkat/L]	12.90 (12.15–13.55)	10.05 (9.50–10.85)	25.10 (24.15–28.40)	15.75 (13.35–16.65)	41.79 <i>p</i> < 0.001*	<i>p</i> ₁₋₂ = 0.091 <i>p</i> ₁₋₃ = 0.001* <i>p</i> ₁₋₄ = 0.893 <i>p</i> ₂₋₃ < 0.001* <i>p</i> ₂₋₄ = 0.005* <i>p</i> ₃₋₄ = 0.091
Blood serum AP [μkat/L]	5.77 (5.42–6.24)	9.40 (8.00–9.75)	15.00 (12.55–15.60)	24.33 (20.86–25.54)	44.09 <i>p</i> < 0.001*	<i>p</i> ₁₋₂ = 0.215 <i>p</i> ₁₋₃ < 0.001* <i>p</i> ₁₋₄ < 0.001* <i>p</i> ₂₋₃ = 0.215 <i>p</i> ₂₋₄ < 0.001* <i>p</i> ₃₋₄ = 0.215
Blood serum MI (ALP/AP)	2.24 (2.10–2.33)	1.13 (1.04–1.26)	1.65 (1.61–2.07)	0.65 (0.59–2.07)	41.51 <i>p</i> < 0.001*	<i>p</i> ₁₋₂ = 0.001* <i>p</i> ₁₋₃ = 0.970 <i>p</i> ₁₋₄ < 0.001* <i>p</i> ₂₋₃ = 0.082 <i>p</i> ₂₋₄ = 0.230 <i>p</i> ₃₋₄ < 0.001*
Periodontal homogenate ALP [μkat/kg]	7.10 (6.60–7.90)	4.25 (3.70–4.95)	4.95 (4.75–5.30)	2.01 (1.82–2.17)	40.75 <i>p</i> < 0.001*	<i>p</i> ₁₋₂ = 0.001* <i>p</i> ₁₋₃ = 0.070 <i>p</i> ₁₋₄ < 0.001* <i>p</i> ₂₋₃ = 0.999 <i>p</i> ₂₋₄ = 0.006* <i>p</i> ₃₋₄ = 0.001*
Periodontal homogenate AP [μkat/kg]	4.18 (3.95–4.23)	8.55 (8.00–9.20)	7.10 (6.35–8.15)	19.19 (15.88–20.11)	43.12 <i>p</i> < 0.001*	<i>p</i> ₁₋₂ < 0.001* <i>p</i> ₁₋₃ = 0.157 <i>p</i> ₁₋₄ < 0.001* <i>p</i> ₂₋₃ = 0.384 <i>p</i> ₂₋₄ = 0.007* <i>p</i> ₃₋₄ < 0.001*
Periodontal homogenate MI (ALP/AP)	1.74 (1.67–1.85)	0.50 (0.47–0.56)	0.71 (0.67–0.80)	0.11 (0.10–0.14)	43.27 <i>p</i> < 0.001*	<i>p</i> ₁₋₂ < 0.001* <i>p</i> ₁₋₃ = 0.166 <i>p</i> ₁₋₄ < 0.001* <i>p</i> ₂₋₃ = 0.348 <i>p</i> ₂₋₄ < 0.001* <i>p</i> ₃₋₄ < 0.001*

Data presented as *Me* (*Q*R). ALP – alkaline phosphatase; AP – acid phosphatase; MI – mineralization index; *p*₁₋₂, *p*₁₋₃, *p*₁₋₄, *p*₂₋₃, *p*₂₋₄, *p*₃₋₄ – probability of differences between the particular groups (numbers in lowercase correspond to group numbers); * statistically significant.

did not change significantly as compared to the control group (*p* = 0.070). In rats with LPS-induced periodontitis combined with chronic thiolactone HHcy, this index decreased 3.5 times as compared to control (*p* < 0.001) and was by 52.7% lower than in the case of LPS-induced periodontitis alone (*p* = 0.006) (Table 2).

Regarding the activity of serum AP in the case of LPS-induced periodontitis, this index increased by 62.9% as compared to the control group, but this change was not statistically significant (*p* = 0.215). In animals with isolated chronic thiolactone HHcy, a significant 2.6-fold increase of AP activity was found as compared to controls (*p* < 0.001). For the combination of LPS-induced periodontitis and chronic thiolactone HHcy, this index significantly increased 4.2 times as compared to control (*p* < 0.001) and was 2.6 times higher than in the case of LPS-induced periodontitis alone (*p* < 0.001) (Table 2).

In periodontal homogenate, AP activity was found to be significantly higher (2.0 times) in rats with LPS-induced periodontitis as compared to controls (*p* < 0.001). In animals with isolated chronic thiolactone HHcy, the periodontal AP activity did not change significantly as compared to the control group (*p* = 0.157). In rats with LPS-induced periodontitis combined with chronic thiolactone HHcy, this index significantly increased 4.6 times as compared to control (*p* < 0.001) and was 2.2 times higher than in the case of LPS-induced periodontitis alone (*p* = 0.007) (Table 2).

An important index that characterizes the condition of bone tissue is the ratio of ALP activity to AP activity, which is defined as MI. The results of our study showed that in the case of LPS-induced periodontitis, the serum MI significantly decreased by 49.6% as compared to the control group (*p* = 0.001). In animals with

isolated chronic thiolactone HHcy, a decrease in MI by 26.3% as compared to control was found, but this change was not statistically significant ($p = 0.970$). In rats with LPS-induced periodontitis combined with chronic thiolactone HHcy, the index decreased 3.4 times as compared to control ($p < 0.001$) and was 42.5% lower than in the case of LPS-induced periodontitis only; however, the latter change was not statistically significant ($p = 0.230$) (Table 2).

Regarding the changes of MI in periodontal homogenate, in rats with LPS-induced periodontitis, this index decreased 3.5 times as compared to the control group ($p < 0.001$). In animals with isolated chronic thiolactone HHcy, a 2.5-fold decrease in the periodontal MI as compared to control was found, but this change was not statistically significant ($p = 0.166$). In rats with LPS-induced periodontitis combined with chronic thiolactone HHcy, the index decreased 15.8 times as compared to control ($p < 0.001$) and was 4.5 times lower than in the case of LPS-induced periodontitis only ($p < 0.001$) (Table 2).

Analyzing the correlation linkages between the serum level of Hcy and the bone metabolism indices, we did not find any significant correlations in group 2 (LPS-induced periodontitis only). At the same time, in rats with LPS-induced periodontitis combined with chronic thiolactone HHcy, a strong direct correlation between the serum Hcy level and the serum AP activity ($r = 0.89$; $p < 0.001$), as well as a moderate direct correlation between the serum Hcy level and the periodontal AP activity ($r = 0.66$; $p = 0.019$) were found. Of particular interest are significant correlations between the serum Hcy level and AP activity in blood serum ($r = 0.89$; $p < 0.001$), AP activity in periodontal homogenate ($r = 0.75$; $p = 0.005$) and MI in blood serum ($r = -0.71$; $p = 0.010$) in animals with isolated chronic thiolactone HHcy (Table 3).

Discussion

The leading role in the pathogenesis of periodontitis is played by the disorders of bone metabolism, which consists in maintaining balance between resorption processes (mediated by osteoclasts) and the formation of bone tissue (mediated by osteoblasts). A number of factors affect and coordinate bone remodeling on both systemic and local levels, ensuring the elimination of micro-damage in the bone matrix, the preservation of bone micro-architectonics and the maintenance of bone strength. The effects of the regulators of bone metabolism are realized through the main signaling pathways of osteoblastogenesis (canonical wingless-beta-catenin (Wnt/ β -catenin)) and osteoclastogenesis (the system of receptor activator for NF- κ B ligand (RANKL)/receptor activator for NF- κ B (RANK)/osteoclastogenesis inhibitory factor (OCIF)).^{20–22}

Among the markers of bone metabolism, the determination of phosphatases is relatively widely studied and used. In bone tissue, ALP is synthesized by osteoblasts and their precursors, and is involved in the mineralization of the bone matrix. The activity of ALP is interpreted as an index of bone tissue formation. Acid phosphatase is a lysosomal enzyme that characterizes the activity of osteoclasts and reflects the processes of bone matrix degradation.

The results of our study showed that LPS-induced periodontitis without comorbid pathology in rats is accompanied by decreases in ALP activity and MI, with a simultaneous increase of AP activity in periodontal homogenate, which indicates diminishing the function of osteoblasts in periodontal bone tissue and the activation of osteoclasts, and hence osteoresorption. Chronic thiolactone HHcy exacerbates the disorders of bone metabolism in the case of periodontitis. It is confirmed by more pronounced changes in all studied indices as compared to animals with LPS-induced periodontitis only. A moderate direct

Table 3. Correlation linkages between the level of homocysteine (Hcy) in blood serum and the indices of bone metabolism in the case of lipopolysaccharide (LPS)-induced periodontitis without comorbid pathology and combined with chronic thiolactone hyperhomocysteinemia (HHcy) (r_{xy})

Parameter	Experimental groups			
	group 2 (periodontitis)	group 3 (HHcy)	group 4 (periodontitis + HHcy)	
Hcy [μ mol/L]	blood serum ALP [μ kat/L]	$r = -0.49$ $p = 0.105$	$r = 0.01$ $p = 0.974$	$r = 0.20$ $p = 0.533$
	periodontal homogenate ALP [μ kat/kg]	$r = -0.19$ $p = 0.564$	$r = -0.08$ $p = 0.814$	$r = -0.10$ $p = 0.755$
	blood serum AP [μ kat/L]	$r = 0.10$ $p = 0.765$	$r = 0.89$ $p < 0.001^*$	$r = 0.89$ $p < 0.001^*$
	periodontal homogenate AP [μ kat/kg]	$r = 0.25$ $p = 0.428$	$r = 0.75$ $p = 0.005^*$	$r = 0.66$ $p = 0.019^*$
	blood serum MI (ALP/AP)	$r = -0.32$ $p = 0.305$	$r = -0.71$ $p = 0.010^*$	$r = -0.51$ $p = 0.092$
	periodontal homogenate MI (ALP/AP)	$r = -0.31$ $p = 0.327$	$r = -0.47$ $p = 0.123$	$r = -0.32$ $p = 0.313$

* statistically significant.

correlation between the serum Hcy level and AP activity in periodontal homogenate ($r = 0.66$; $p = 0.019$) indicates the association between Hcy and osteoclast activity in the case of experimental periodontitis.

Vacek et al. suggest that in addition to the direct Hcy action on the bone matrix, Hcy can modulate bone remodeling via increased osteoclast activity and decreased osteoblast activity.²³

Homocysteine can also directly affect osteoclast performance. Homocysteine has been demonstrated to exert a potent stimulatory influence on oxidant signaling, while osteoclasts are known to be sensitive to elevated reactive oxygen species (ROS) levels.²⁴ In vitro experiments enriching growth media with Hcy for the bone marrow cell culture demonstrated that in these cells, the upregulation of the formation of osteoclasts and the downregulation of apoptosis were caused by an increased production of ROS.²⁵ Elevated Hcy also disrupts balance between the phosphorylation and dephosphorylation of protein kinases (PKs) modulating bone cell remodeling; this can cause the cell-wide disruption of molecular mechanisms in bone marrow-derived osteoclasts. For instance, Hcy has been shown to increase the phosphorylation of P38 mitogen-activated protein kinases (MAPKs) mediated by RANKL.²⁵

Patients with HHcy show a higher risk of fractures due to decreased bone mineral density, as a result of bone resorption caused by elevated osteoclast activity.²⁶ Additionally, in human bone marrow stromal cells, elevated Hcy levels activate the caspase-dependent apoptosis pathway, which also results in compromised bone repair.²⁷ Bone resorption is slowed down in unfavorable oxidizing environments, which are activated by Hcy binding as a ligand to peroxisome proliferator-activated receptor gamma (PPAR- γ) expressed in bone cells.²⁸ A study done on a HHcy mouse model showed the attenuation of PPAR- γ , tissue inhibitor of matrix metalloproteinase 4 (TIMP-4) and thioredoxin (an antioxidant), while inducing matrix metalloproteinase 9 (MMP-9), TIMP-3, and nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase 4 (Nox4).²⁹

A correlation between Hcy and the bone turnover markers detected in serum, such as deoxypyridinoline³⁰ and C-terminal telopeptide of type I collagen,³¹ was found in some studies.

However, the effect of Hcy on osteoblast activity is comparatively little understood. Osteoblasts are involved in bone formation, and in vitro studies show that elevated concentrations of Hcy result in a moderate increase in primary human osteoblast activity.³² However, the extent of this effect is less prominent than the effects on primary human osteoclasts described above. This indicates the lack of balance between osteoblast and osteoclast activity. In the HS-5 cell line, Hcy has been shown to induce apoptosis in NF- κ B-activated primary human bone marrow stromal cells through the ROS-mediated

mitochondrial pathway.²⁶ The resulting escape of cytochrome c from the mitochondria activates caspases 3 and 9, and is likely to produce an apoptotic effect on osteoblasts. In a study by Herrmann et al., the inhibition of osteoblast activity was supported by the reduced circulating osteocalcin level (by 40%) observed in rats with HHcy compared to controls.³³

In another research by Herrmann et al., the bone tissue of rats with HHcy showed an increased accumulation of Hcy, with 65% of it bound to collagen of the extracellular matrix.³⁴ The accumulation of Hcy produced a “spongy” bone appearance and resulted in decreased bone strength.³⁴ This bone-specific accumulation of Hcy is a mechanism likely to underlie the detrimental effects of HHcy on bone tissue. Furthermore, Hcy has been found to downregulate the mRNA expression of protein-lysine 6-oxidase (LOX), an enzyme essential for cross-linking in collagen.³⁵

Limitations

There are some limitations to this study. Firstly, the study sample size was small; therefore, the results are presented as preliminary. Bone metabolism was only determined with the use of biochemical markers, without imaging methods, such as computed tomography (CT) scanning. Further investigations are needed to explore the clinical implications of these findings.

Conclusions

The disruption of bone remodeling by LPS-induced periodontitis results in imbalance between bone matrix synthesis by osteoblasts and bone turnover by osteoclasts. Moreover, chronic thiolactone HHcy enhances the violations of bone metabolism in LPS-induced periodontitis. The osteotoxic effect of HHcy is associated with the activation of osteoclastogenesis and enhanced bone resorption. However, it is likely that there are other mechanisms of negative effects of HHcy on bone metabolism, which require further research.

Ethics approval and consent to participate

The experimental procedures including animal treatment fully complied with relevant regulations. The experimental design and protocol were approved by the Bioethics Committee of I. Horbachevsky Ternopil National Medical University (protocol No. 64 of May 17, 2021).


Data availability


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


Consent for publication


Not applicable.

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YouTube™ as a source of information on vitamin D: A content-quality analysis

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Abstract

Background. YouTube™ is considered a convenient and popular platform to seek healthcare information, which is uploaded by people of various professions.

Objectives. This study aimed to assess the content and the quality of the YouTube videos containing information about vitamin D for children.

Material and methods. A YouTube search was performed using the terms ‘vitamin D for children’ and ‘vitamin D deficiency in children’. Two independent reviewers scored the videos with the use of a customized 11-point scoring scheme and the 5-point Global Quality Scale (GQS) to assess the content and the quality of each video. The videos were further classified into poor, moderate and good videos. The Kruskal–Wallis and χ^2 tests as well as Spearman’s correlation coefficient were used for statistical analysis.

Results. In a total of 120 videos screened, 70 videos were included. They had a median content score of 4 and a median GQS score of 3. According to the information included in the videos and their overall quality, most of the videos were categorized as moderate ($n = 33$; 47.14% and $n = 32$; 45.71%, respectively). Good-quality videos had statistically significantly higher content scores as compared to the other groups ($p = 0.001$). A positive correlation was found between the content score and the GQS score ($\rho = 0.434$; $p = 0.001$).

Conclusions. Most of the videos on YouTube about vitamin D were rated as moderate according to the sufficiency of the information and also their overall quality. This suggests that health organizations need to make further efforts to create good-quality medical content on popular social media platforms, such as YouTube.

Keywords: vitamin D deficiency, social media, YouTube™, vitamin D

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Introduction

Vitamin D has become a globally popular topic and its deficiency is recognized as a major worldwide public health concern.¹ Vitamin D plays an important role in the human body, maintaining the normal growth and mineralization of the bone and other calcified tissues, including the teeth.² It has been proven to be beneficial for general human well-being.³ The main source of vitamin D is exposure to natural sunlight whereby the ultraviolet rays that strike the skin trigger the synthesis of vitamin D.⁴ Vitamin D is also naturally present in some foods and is available as a dietary supplement.⁵

The vitamin D status is classified based on the level of 25-hydroxyvitamin D (25 (OH)D) in the serum. The U.S. Endocrine Society defines vitamin D deficiency as 25(OH)D <20 ng/mL (<50 nmol/L), insufficiency as 21–29 ng/mL (52.5–72.5 nmol/L) and sufficiency as >30 ng/mL (>75 nmol/L).⁴ Vitamin D deficiency is often asymptomatic; however, severe and prolonged deficiency can manifest symptoms, including muscle aches, bone pain, proximal muscle weakness, and in the long run, it can cause bone mineralization diseases, such as rickets in children and osteomalacia in adults.⁴ It has also been associated with bone fractures, falls, functional limitations, some types of cancer, diabetes, and cardiovascular disease.⁴ However, the association of these diseases with vitamin D deficiency is still unclear.

The major cause of vitamin D deficiency is the lack of sun exposure.⁴ Therefore, individuals with limited sun exposure are at risk of having vitamin D deficiency. Besides that, individuals who are dark-skinned, obese or undernourished are also at risk of having a low vitamin D level. These groups of individuals are recommended to do screening for vitamin D deficiency and vitamin D supplements might be considered for those with confirmed vitamin D deficiency.⁴

According to the American Association of Clinical Endocrinology (AACE), the recommended daily intake of vitamin D to prevent vitamin D deficiency and to maximize bone health is 400 IU for infants and 600 IU for children aged 1–18 years; as for adults, the recommended daily intake of vitamin D is 600 IU for adults between 19 and 70 years of age and 800 IU for adults older than 70 years.⁴

There has been an increasing trend in the use of social media throughout the years. YouTube™ has been ranked as the second most popular social media platform with 1.9 billion active users monthly in 2018.⁶ Moreover, in 2019, statistics showed that among the 7.7 billion population in the world,⁷ 45% (3.5 billion) are the active users of social media.⁸ A study by Orr revealed that YouTube was the world's biggest video sharing and hosting site on the Internet.⁹ Hence, it has been suggested that YouTube is considered as a convenient and popular platform for the public to seek further healthcare knowledge as well as advice and assistance.⁹

Several studies have been conducted to ascertain the quality of information that is offered by YouTube videos in various medical healthcare disciplines, including the dental aspects.^{10–13} Erroneous and deceptive health-related information has been reported, and as such it might harm the public's health. To the best of our knowledge, there is no study on social media regarding the evaluation of information on vitamin D deficiency in children. Therefore, the content and the quality of videos on YouTube regarding this topic was evaluated in this study in order to determine if these videos provide parents and users with adequate and accurate information.

Methodology

Ethics approval was granted by the Human Ethics Committee at the MARA University of Technology (Universiti Teknologi MARA), Shah Alam, Malaysia, to conduct this study using the publicly available data on the Internet.

Video collection

The structured method used in previous studies was systematically implemented in this study to search the contents of YouTube.^{12–14} The Google Trends application was used to identify the search terms that are most frequently used on the Internet with regard to the topic discussed in this study.¹⁵ In this research, 2 search terms were used, namely 'vitamin D for children' and 'vitamin D deficiency in children.' The search was carried out on March 2, 2020, using YouTube default settings without any filters. A previous study reported that a vast majority of YouTube users went through the first 30 videos multiple times in a day.¹⁶ Hence, videos that appeared on the first 3 pages (the first 60 videos) for each search term were included in the study. Meanwhile, the layout of the site was changed. Therefore, the first 60 videos that appeared on the screen for each term were counted and explored. These 120 videos were downloaded and briefly scanned to exclude irrelevant videos, videos that were not in English and duplicates with regard to the 2 search terms before further evaluation.

Video evaluation

The demographics of the videos, such as the title, the country of origin, duration in minutes, the date of upload, the source of upload, and the total number of views, likes and dislikes, were extracted. The sources of upload were categorized as academic or professional organizations, companies, healthcare professionals, individual users, and others. Viewer interaction was evaluated in terms of the interaction index and the viewing rate, which were calculated for

each video based on the methods described in previous studies (Equations 1,2).^{13,14}

$$\text{interaction index} = \frac{\text{number of likes} - \text{number of dislikes}}{\text{total number of views}} \times 100\% \quad (1)$$

$$\text{viewing rate} = \frac{\text{number of views}}{\text{number of days since upload}} \times 100\% \quad (2)$$

These videos were further evaluated independently by 2 researchers using a customized 11-point scoring scheme that was constructed with reference to the 2019 AACE guidelines on vitamin D deficiency (Table 1).⁴ Each video was scored from 0 to 11 with regard to providing parents with information about vitamin D for their children in terms of its importance, sources, the recommended daily intake, risk factors, and the consequences of vitamin D deficiency for the child's health. Each aspect mentioned in the video correctly was given 1 point, and if the aspect was not mentioned or incorrect information was given, then the score was 0 (Table 1).¹⁴ Videos with a score of 0–3 were evaluated as having 'poor information content', a score between 4 and 7 was considered as 'moderate information content' and a score between 8 and 11 was considered as 'good information content'.

As previously reported,^{12,13} the overall video quality was evaluated based on the 5-point Global Quality Scale (GQS) (a range of 1–5, with 1 indicating poor quality and 5 indicating excellent quality) (Table 2). The scores 1 and 2 were categorized as 'poor quality', a score of 3 was categorized as 'moderate quality', while 4 and 5 were categorized as 'good quality'. These scores were tabulated for all videos on both search terms and the scores from the 2 researchers were compared. Videos with different scores were then re-evaluated by reviewing the literature and

Table 2. Global Quality Scale (GQS) criteria used to score the quality of videos about vitamin D

GQS	Description
1	poor quality and flow, most information missing, not at all useful for patients
2	generally poor quality and poor flow, some information listed, but many important topics missing, of very limited use to patients
3	moderate quality, suboptimal flow, some important information adequately discussed, but other poorly discussed, somewhat useful for patients
4	good quality and generally good flow, most of the relevant information listed, but some topics not covered, useful for patients
5	excellent quality and flow, very useful for patients

discussing the issue by the 2 researchers until consensus was achieved. A new score was then given based on the agreed consensus.¹³

Statistical analysis

The data was entered in a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, USA). The statistical analysis was performed with the use of the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). Descriptive statistics referred to the characteristics of the videos, comprising the number of views, the number of likes, the number of dislikes, duration in minutes, the number of days since upload, the viewing rate, and the interaction index. Non-parametric tests were performed after testing the normality of data distribution with the Shapiro–Wilk test. Continuous variables were presented as median and interquartile range (*Me (IQR)*), while categorical variables were expressed as frequency and percentage (*n (%)*). The Kruskal–Wallis test for continuous variables and the χ^2 test for categorical variables were used to compare the video parameters between poor, moderate and good information content as well as between the GQS categories. The Spearman rank-order

Table 1. Content criteria used to score the videos containing information about vitamin D

Content criteria	Description	Score
Importance of vitamin D	– growth – mineralization of bone and the teeth	1
Sources of vitamin D	– sunlight exposure – food – food fortified with vitamin D – supplements	1 each (total of 4)
Recommended daily intakes of vitamin D to prevent vitamin D deficiency and maximize bone health	– 400 IU for infants – 600 IU for children to adults – 800 IU for adults above 70 years of age	1
Risk factors for vitamin D deficiency	– limited sun exposure – malnutrition/malabsorption – obesity – dark skin	1 each (total of 4)
Health effects of vitamin D deficiency	– rickets – bone fractures	1
Total content score		11

correlation coefficient was used to investigate the correlation between video characteristics and the total content and GQS scores. The interclass correlation coefficient (ICC) was calculated to determine the degree of agreement between the 2 raters (model: two-way mixed; type: consistency).¹² A p -value <0.05 was considered statistically significant.

Results

The initial search using the terms 'vitamin D for children' and 'vitamin D deficiency in children' revealed a total of 120 videos, of which 70 were included, whereas 50 were excluded due to the reasons provided in Fig. 1. The analysis of these videos suggested that most of them were dedicated to all age groups. Hence, the videos were analyzed regardless of the age group.

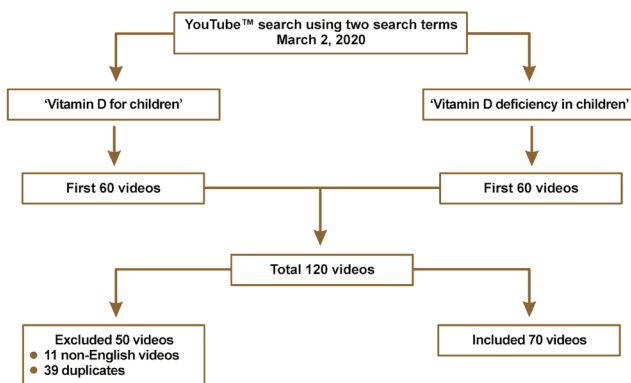


Fig. 1. Flow diagram of YouTube videos search strategy

Demographics

The descriptive statistics of the videos are presented in Table 3. The median duration of the videos was 2.75 min. The total number of views was 6,479,549 with a median

Table 3. Demographic characteristics of the included videos

Characteristics	Me (IQR)	Minimum	Maximum
Number of views	2,984 (14,256)	29	2,780,250
Number of likes	17 (89)	0	34,000
Number of dislikes	1 (7)	0	525
Duration [min]	2.75 (3.76)	0.12	50.55
Number of days since upload	1,469 (1,920)	55	4,190
Viewing rate	2.25 (21.75)	0.02	1,408.72
Interaction index	0.53 (0.69)	-0.75	6.14
Content score	4 (4)	0	11
GQS score	3(1)	1	5

Me – median; IQR – interquartile range.

viewing rate of 2.25 views/day. The median distribution of the interaction with viewers was 17 likes and 1 dislike with a median viewer's interaction index of 0.53. The median content score was 4 and the median GQS score was 3. Most of the viewed videos were uploaded by academic institutions and professional organizations ($n = 27$; 38.57%), whereas other videos were uploaded by companies, healthcare professionals, individual users, and others (Fig. 2).

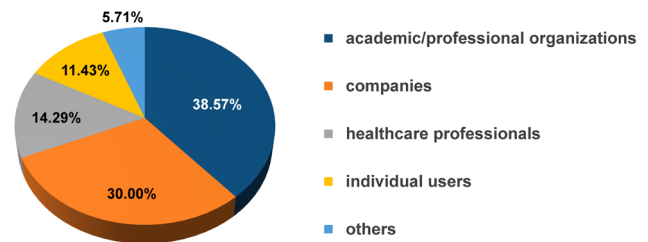


Fig. 2. Distribution of the videos according to the video source

Content of the videos

The videos contained a variety of information about vitamin D in terms of its importance, sources, the recommended daily intake, risk factors, and the health effects of vitamin D deficiency. The majority of the videos addressed the sources of vitamin D ($n = 62$; 88.57%), followed by the importance of vitamin D for the body ($n = 41$; 58.57%). Less than half of the videos discussed the health effects of vitamin D deficiency ($n = 31$; 44.29%) and risk factors for vitamin D deficiency ($n = 33$; 47.14%). Only 37.14% ($n = 26$) of the videos mentioned the recommended daily intake to prevent vitamin D deficiency and to maximize bone health (Fig. 3). As far as the evaluation of content is concerned, a total of 26 videos (37.14%) were defined as poor, 33 (47.14%) as moderate and 11 videos (15.71%) as good (Table 4). Good-content videos had a significantly longer duration ($p = 0.040$) and higher GQS scores ($p = 0.001$) than the other groups. Furthermore, most of the good-content videos were uploaded by aca-

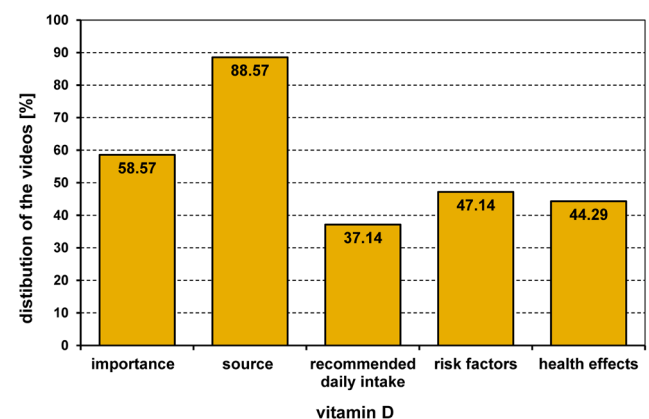


Fig. 3. Distribution of the videos according to the content criteria

Table 4. Comparison of video characteristics between good-, moderate- and poor-content videos

Characteristics	Content			p-value	
	poor n = 26	moderate n = 33	good n = 11		
Number of views M ±SD	2,086 (14,427)	3,832 (45,506)	2,990 (9,983)	0.740	
Number of likes M ±SD	8 (96)	17 (619)	38 (56)	0.710	
Number of dislikes M ±SD	1 (7)	2 (18)	1 (6)	0.750	
Duration [min] M ±SD	2.19 (3.34)	3.13 (2.99)	5.36 (21.29)	0.040*	
Number of days since upload M ±SD	1,469 (1,479)	1,173 (2,347)	1,798 (1,799)	0.510	
Viewing rate M ±SD	1.46 (16.64)	3.27 (44.10)	2.25 (3.74)	0.500	
Interaction index M ±SD	0.53 (0.66)	0.53 (1.21)	0.56 (0.54)	0.890	
Source of upload n (%)	academic/professional organizations	8 (29.6)	15 (55.6)	4 (14.8)	0.004*
	companies	10 (47.6)	8 (38.1)	3 (14.3)	
	healthcare professionals	4 (40.0)	5 (50.0)	1 (10.0)	
	individual users	3 (37.5)	3 (37.5)	2 (25.0)	
	others	1 (25.0)	2 (50.0)	1 (25.0)	
GQS score M ±SD	3 (1)	3 (1)	4 (1)	0.001*	

Kruskal–Wallis test for continuous variables and χ^2 test for categorical variables. * statistically significant.

demic institutions and professional organizations ($n = 4$; 14.8%), whereas most of the poor-content videos were uploaded by companies ($n = 10$; 47.6%), with the difference being statistically significant ($p = 0.004$). However, with regard to the number of views, likes and dislikes, the number of days since upload, the viewing rate, and the interaction index, no significant differences were observed among the groups ($p > 0.05$) (Table 4).

Quality of the videos

The overall quality of the videos was assessed using GQS. With regard to quality, a total of 13 videos (18.57%) were scored poor, 32 (45.71%) were scored moderate and 25 (35.71%) were scored good (Table 5). Good-quality videos had significantly higher information content scores than the other groups ($p = 0.001$). Most of the good-quality videos ($n = 10$; 37.0%) were uploaded by academic institutions and professional organizations, whereas most of the poor-quality videos were equally uploaded by healthcare professionals ($n = 5$; 50.0%) and academic institutions and professional organizations ($n = 5$; 18.5%), with significant differences between poor- and good-quality videos ($p = 0.001$). However, with regard to the number of views, likes and dislikes, duration, the number of days since upload, the viewing rate, and the interaction index, no significant differences were observed among the groups ($p > 0.05$) (Table 5).

Correlation analysis

The correlations of the descriptive characteristics of the evaluated videos with the total content and GQS scores was examined. No significant correlations were identified between the video characteristics and the total content score except for the duration of the video ($\rho = 0.318$; $p = 0.007$). Likewise, no significant correlations between the video characteristics and the video quality were observed. However, a weak positive correlation was found between the total content score and the GQS score ($\rho = 0.434$; $p = 0.001$). The interclass correlation coefficient was calculated for the content score (0.96) and the GQS score (0.76), indicating an excellent and good agreement, respectively.

Discussion

Vitamin D deficiency has been highlighted as a serious public health concern.¹ The increasing popularity of social media and the Internet has attracted a significant proportion of the population to search for health information or advice/assistance on medical problems through YouTube. The validity of the information on YouTube is questionable due to its non-standardized content and simple video sharing without any quality control.¹⁷ This study aimed to assess the content and quality of videos about

Table 5. Comparison of video characteristics between good-, moderate- and poor-quality videos

Characteristics	Quality			p-value	
	poor n = 13	moderate n = 32	good n = 25		
Number of views M ±SD	1,962 (51,440)	3,779 (10,143)	3,405 (24,815)	0.900	
Number of likes M ±SD	7 (237)	10 (73)	38 (89)	0.670	
Number of dislikes M ±SD	1 (110)	1 (5)	3 (14)	0.700	
Duration [min] M ±SD	2.15 (4.41)	2.46 (2.88)	3.25 (5.94)	0.250	
Number of days since upload M ±SD	1,663 (1,530)	1,482 (2,367)	1,142 (1,520)	0.350	
Viewing rate M ±SD	0.96 (146.30)	1.95 (7.07)	3.05 (38.11)	0.490	
Interaction index M ±SD	0.33 (1.14)	0.42 (0.65)	0.63 (0.77)	0.230	
Source of upload n (%)	academic/professional organizations	5 (18.5)	12 (44.4)	10 (37.0)	0.001*
	companies	3 (14.3)	9 (42.9)	9 (42.9)	
	healthcare professionals	5 (50.0)	3 (30.0)	2 (20.0)	
	individual users	0 (0.00)	6 (75.0)	2 (25.0)	
	others	0 (0.00)	2 (50.0)	2 (50.0)	
Content score M ±SD	2 (2)	5 (3)	9 (2)	0.001*	

Kruskal–Wallis test for continuous variables and χ^2 test for categorical variables. * statistically significant.

vitamin D on YouTube. Although numerous studies have assessed the content of YouTube videos and their quality with regard to different medical aspects, this is the first study to evaluate the content and the quality of the YouTube videos related to vitamin D. The findings showed that YouTube videos on vitamin D health-related information had different content and quality. There were only a few videos about vitamin D with good content and good quality on YouTube.

Several studies have suggested a role for vitamin D for health outcomes,^{18–20} including maintaining the normal growth and mineralization of the bone and other calcified tissues, such as the teeth.^{2,21} Vitamin D also has been proven to be beneficial for general well-being,³ to prevent rickets and to boost the immune system. The findings of the current study showed that more than half of the videos ($n = 41$; 58.57%) advocated the importance of vitamin D and its association with health benefits for the bone and the teeth, normal growth, calcium absorption, and rickets prevention. Only a few videos provided information on the role of vitamin D in boosting the immune system.

Most of the videos ($n = 62$; 88.57%) mentioned the sources of vitamin D, including the exposure of the skin to natural sunlight and common dietary foods, such as cod liver oil, fatty fish, eggs, milk/dairy products, mushrooms, and vitamin D-fortified food. However, the contribution of the food sources in achieving the appropriate vitamin

D levels is considered low as compared to sunlight exposure. Likewise, supplements are one of the alternatives to obtain vitamin D.⁵

Behavior and attitudes toward exposure to sunlight, obesity, skin pigmentation, age, gender, dairy allergy, cloudy weather, and pollution are the main determinants of the status of vitamin D, and are considered risk factors for vitamin D deficiency.^{21,22} However, only 33 videos (47.14%) addressed the factors hindering the production of vitamin D and leading to its deficiency in the body.

A mild lack of vitamin D may not cause symptoms, but can result in tiredness, and general aches and pains. A more severe lack can cause serious problems, such as rickets in children and osteomalacia in adults.²³ Nevertheless, less than half of the videos ($n = 31$, 44.29%) highlighted the health effects of vitamin D deficiency, such as the impaired mineralization of bone.

Some people are more at risk of vitamin D deficiency; hence, they are recommended to take vitamin D supplements routinely. These groups of people include children below 5 years of age, pregnant and breastfeeding women, seniors above 65 years of age, and those who are not exposed to the sun. Vitamin D supplementation is also recommended for people with darker skin and for those with certain gut, liver or kidney diseases.²³

The U.S. Institute of Medicine recommends that dark-skinned infants who are exclusively breastfed and are at greater risk of rickets should receive 400 IU of vitamin D

supplement per day.⁴ Healthy children and adults should take 600 IU per day, whereas adults older than 70 years should take 800 IU per day to maintain skeletal health.⁴ Vitamin D supplementation in these ranges is likely to prevent bone loss, may improve bone density, may reduce fractures, and appears to reduce falls. Most videos ($n = 44$; 62.86%) failed to address the recommended daily intake/consumption of vitamin D to maximize bone health and prevent vitamin D deficiency.

As reported in the literature, a considerable proportion of parents rely on the Internet-based sources to retrieve information regarding vitamin D for their children.^{5,24} Parental health awareness and practices usually have a direct effect on their child's health.⁵ Therefore, increasing knowledge about vitamin D among parents is vital to achieve and maintain adequate levels of vitamin D in children.²¹

A study by Day et al. stated that around half of the parents reported the lack of information about vitamin D for their young children and the majority obtained information by proactively seeking it via a range of medical and parenting websites/platforms, including YouTube.²⁴ Additionally, parents also declared to improve the information about vitamin D through signposting to the approved websites, such as hospital, medical and parenting websites, and also through e-mails, text messages, smartphone Apps, an online messaging service with the assistance of a health professional, and YouTube videos.²⁴ These findings suggest that the YouTube platform can be considered as a potential information source for parents. However, our findings revealed that the content of most videos analyzed in this study was moderate and lacked some information.

Although some demographics of the analyzed videos, i.e., the number of likes, the number of dislikes, the viewing rate, and the interaction index, showed a positive correlation with the total content score, the duration of the video duration was the only parameter that significantly correlated with the total content score ($\rho = 0.318$; $p = 0.007$). This is similar to previous studies that revealed a positive correlation between the information content and duration of YouTube videos.^{25,26} Likewise, the present study demonstrated that good-content videos had a longer duration, a greater number of likes, fewer dislikes, and a greater interaction index than videos in the moderate- and poor-content groups. However, among the variables, only the duration of the video turned to be statistically significantly different ($p = 0.040$). This indicates that viewers had a positive interaction with the well-organized and educational content of videos about the importance of vitamin D for well-being, and such videos were found to have an adequate (longer) duration (Me : 5.36 min) as compared to poor-content videos (Me : 2.19 min). This finding suggests that videos on the topic of vitamin D should include informative and advanced content, and be produced at reasonable time to gain larger audience on the YouTube platform.

According to the median GQS scores obtained in this study, most of the evaluated videos were generally of moderate quality. Good-content videos had significantly higher GQS scores than the other groups, and a weak positive correlation was observed between the quality score and the content score. Other studies evaluating YouTube videos indicated a moderate positive correlation between the video's quality and content.^{27,28} The information flow and a great number of informative elements in the videos, such as images and video captions, increased the quality and content scores. In addition, videos that contained balanced information and were uploaded/published by trustworthy sources were more beneficial for viewers/users. These might elucidate the correlation between the quality score and the content score.

Strengths and limitations

There are several limitations of this study. Firstly, only English-language videos were analyzed; hence, 11 videos were excluded. It may have limited the results by ignoring the popularity of this topic in developing countries, in which English is not widely used. Secondly, the YouTube platform is very dynamic, and the results may change when new videos are uploaded or the old ones are removed. This study reveals the information about videos on the topic of vitamin D at a particular time. Therefore, our results depended on the time at which the search took place. Finally, even though scientific criteria from the current guidelines were used, it was subjective to evaluate the videos with the use of a customized information content scoring scheme by 2 researchers, as there is no validated tool for assessing video content to date. Despite the limitations, this method can still be used to obtain preliminary information that can be considered reliable regardless of its relatively subjective nature, and such an approach has been used in several studies.^{14,26} Moreover, according to the obtained ICC values, the agreement between the 2 researchers was excellent and good, thus indicating that the method of evaluation of video content was reliable. The small number of good-content videos, as observed in the current and previous studies, points to the need for professionals to be more active on such platforms and to improve the quality of education through these media.

Conclusions

YouTube is continuously being accessed and it offers a wide diversity of health-related information. Although most of the videos on vitamin D were rated as moderate with regard to the sufficiency of information as well as quality, the results of this study disclosed that YouTube videos still could not be considered as an excellent source of information for parents on vitamin D for their children. Health organizations need to make further efforts to cre-

ate good-quality medical content on popular social media platforms, such as YouTube. Further studies evaluating the content and the quality of videos in different languages and using other social media are warranted.

Ethics approval and consent to participate

Not applicable.


Data availability


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

Consent for publication


Not applicable.


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Effects of botulinum toxin in patients with myofascial pain related to temporomandibular joint disorders: A systematic review

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Abstract

Botulinum toxin is used as an alternative for the treatment of chronic refractory myofascial pain derived from temporomandibular disorders (TMDs). It is important to establish the benefits of botulinum toxin in this type of symptomatology. The aim of the study was to conduct a systematic review in order to evaluate the effects of botulinum toxin in patients with myofascial pain related to temporomandibular disorders. The search was carried out systematically, without limitations of language or year of publication, until February 2021. The databases searched included PubMed, Web of Science, Scopus, The Cochrane Library, and Latin American and Caribbean Health Sciences Literature (LILACS). Partial gray literature was searched using Google Scholar, ClinicalTrials.gov, OpenGrey, and the reference lists of selected articles. Randomized controlled clinical trials evaluating the effects of botulinum toxin in the treatment of myofascial pain were included. The risk of bias was assessed with the Cochrane RoB 2.0 tool, and the The Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach was used to determine the certainty of the scientific evidence. A total of 900 studies were retrieved, out of which only 8 randomized clinical trials were selected. From these 8 studies, the data of a total of 314 patients, predominantly women, between the ages of 18 to 75 years was obtained. After the assessment of the studies with the RoB 2.0 tool, 7 studies showed some concerns regarding the reported results and only one was at a low risk of overall bias. The analysis of the studies has shown that low doses of botulinum toxin are effective in the treatment of refractory myofascial pain associated with temporomandibular disorders. The studies presented medium- to low-certainty evidence.

Keywords: botulinum toxin, myofascial pain, temporomandibular joint disorders

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Introduction

The American Academy of Orofacial Pain (AAOP) defines temporomandibular disorder (TMD) as a generic term that contains a series of clinical problems that affect the temporomandibular joint, masticatory muscles and associated structures.^{1,2} Temporomandibular disorder has a multifactorial etiology that involves biological, infectious, hormonal, psychological, social, and emotional factors.^{3–10}

Patients with TMD present with pain,^{11–20} limitation of movement, dysfunction, fatigue, subjective weakness, and stiffness of the facial and chewing muscles.²¹ In addition, there is a subgroup within the TMD that corresponds to musculoskeletal disorders, the most prevalent of which are localized myalgia and myofascial pain.¹⁷ Myofascial pain can present with acute to moderate intensity and is characterized by the presence of sensitive areas called trigger points, located in bands, tendons and muscle fascicles, and generating deep and localized pain in the tense muscular band. However, pain can also occur in other areas distant from the trigger point.^{17,21–23}

Different treatment approaches have been proposed including conservative therapies such as pharmacotherapy,^{4,24–26} physical therapy,^{14,27,28} ultrasound, transcutaneous electrical nerve stimulation,¹⁷ occlusal therapy (occlusal splints),^{29–32} and psychotherapy.^{26,33–35} On the other hand, more invasive procedures such as dry needling and acupuncture are also available.¹⁷ However, even after receiving these treatments, the symptoms may partially persist. In this chronic condition, botulinum toxin type A (BTX-A) has recently been used as the alternative for the longer relief of the symptoms of chronic refractory myofascial pain.^{20,28,36,37,38}

The BTX-A is an exotoxin synthesized by a spore-forming gram-negative anaerobic bacterium called *Clostridium botulinum*. This powerful botulinum neurotoxin performs its action at the presynaptic junction of alpha and gamma motor neurons by blocking Ca.³⁵ It has a dual mechanism of action on the neuromuscular junction such as the inhibition of acetylcholine exocytosis from the nerve end plates (temporary weakening of nerve endings and consequent relaxation of muscle contraction or paralysis, depending on the dose, without any systemic effect) and the inhibition of the release of substance P and glutamate to reduce inflammatory pain.³⁹

Despite the aforementioned factors, there is still a lack of unification and collection of scientific information on the benefits of BTX-A therapy. Therefore, the objective of this systematic review was to assess the effects of botulinum toxin in patients with myofascial pain related to TMD.

Material and methods

Protocol and registration

The present review was carried out following The Cochrane Handbook for Systematic Reviews of Interventions,⁴⁰ and reported as suggested using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.⁴¹ Likewise, the protocol of the study was registered in the PROSPERO Centre for Reviews and Dissemination (CRD) database at the University of York (Heslington, UK), and the National Institute for Health and Care Research (London, UK),⁴² under the number: CRD42020168889.

Eligibility criteria

To define the eligibility criteria, the acronym PICOS (Population, Intervention, Comparison, Outcomes and Study design) was used:

- **Population** – adult patients with TMD-related myofascial pain;
- **Intervention** – botulinum toxin injection treatment for myofascial pain;
- **Comparison** – no treatment, placebo or other specific treatment including physical therapy, occlusal splints, drug therapy, or acupuncture;
- **Primary Outcome** – changes in intensity of myofascial pain due to botulinum toxin treatment;
- **Effect measures** – changes from baseline to last available tracking measured by visual analogue scales or similar tools; and
- **Study design** – randomized clinical trials (RCTs) conducted in humans.

The exclusion criteria included studies in children or adolescents, studies on craniofacial anomalies or neuromuscular diseases, literature or systematic reviews, letters to the editor, pilot studies, case report studies, in vitro studies, and animal studies.

Information sources, search strategy and study selection

The search for studies was carried out regardless of the language or year of publication in following electronic databases: MedLine (via PubMed), Scopus, The Cochrane Library, Latin American and Caribbean Health Sciences Literature (LILACS), Embase, Web of Science. Additionally, a partial search of gray literature using specific keywords was conducted up to February 2021 (the detailed data is available from the corresponding author on reasonable request), using Google Scholar, OpenGrey and ClinicalTrials.gov databases. The first 100 records were searched using

Google Scholar. Also, the reference lists of the selected articles were checked to ensure that no potential articles were lost. Articles were managed using EndNote software (Thomson Reuters EndNote X7®; New York, USA) to avoid possible duplication.

The study selection was carried out independently in 2 phases by 2 reviewers (RMRH and KJAS). In the first phase, the reviewers screened titles and abstracts identified from the results of the electronic database and additional sources. Then, studies with titles and abstracts that did not meet the inclusion criteria and duplicate studies were removed. In the second phase, full-text studies were retrieved to confirm their eligibility, according to the inclusion criteria. The reference lists of the selected articles were also evaluated. The reviewers independently selected articles for inclusion in a qualitative synthesis. Disagreements were resolved by verbal discussion and consensus was reached with the help of a third reviewer (LEAG), when necessary.

Data collection process and data elements

Two reviewers (RMRH and KJAS) independently extracted data from included studies using a standardized Excel spreadsheet. The following data was extracted: study design, sex and age of the patient, sample size, diagnosis of TMD, diagnosis of myofascial pain, muscles involved, treatment approach, area of application of botulinum toxin, number of doses, and treatment time. At the beginning of the study, we planned to include the results on the quality of life of the patients. However, this was not possible as none of the studies selected for the review assessed this variable. To clarify and resolve doubts about the studies, we contacted the authors by e-mail.

Risk of bias in individual studies

The risk of bias (RoB) assessment of the RCTs was performed using the Cochrane Risk of Bias tool (RoB 2.0; Cochrane, London, UK).⁴³ The following domains were considered: randomization process, deviations from planned interventions, missing outcome data, outcome measurement, and selection of the reported result. Each domain was assessed as having: a low risk of bias, some concerns or a high risk of bias. Then, an overall RoB judgment was assigned to each study as: low risk (if all domains had a low RoB), some concerns (if in at least one domain there had been some concerns) or high risk (if in one or more domains there had been some concerns).⁴³

In addition, the degree of certainty of the evidence in the studies was assessed using The Grading of Recommendations Assessment, Development and Evalua-

tion (GRADE) approach⁴⁴ according to the categories (high, moderate, low, and very low).

Both reviewers (RMRH and KJAS) independently assessed the risk of bias and the certainty of the evidence from the included studies. Discrepancies were resolved by verbal discussion and consultation with the third reviewer (LEAG).

Summary measures

Primary outcome measures were based on quantitative data (efficacy of botulinum toxin therapy in TMD-related myofascial pain after botulinum toxin injection). Mean differences and 95% confidence intervals (CIs) for changes were assessed based on patient responses, using a visual analogue scale (VAS).

Synthesis of the results

The data collected from the included studies was synthesized and analyzed in a description table. After the evaluation and taking into account the differences between the botulinum toxin injection protocols, the sample size, the doses, and the follow-up periods, it was considered that the methodology of the studies was not homogeneous. For this reason, a meta-analysis was not performed.

Results

Study selection

In the first phase of the search strategy, a total of 787 studies were identified in the electronic databases (570 in PubMed, 5 in Scopus, 24 in LILACS, 15 in Embase, 60 in Web of Science, and 113 in the Cochrane Library). Furthermore, 113 studies were found during the partial search of the gray literature (100 on Google Scholar, 13 on ClinicalTrials.gov) published from 2008 to 2020. No studies were retrieved from OpenGrey or reference lists. A total of 890 studies were eliminated for being duplicated and not meeting the eligibility criteria after reading the titles and abstracts. Ten studies were obtained for full-text assessment according to the inclusion and exclusion criteria. Two studies were excluded, of which 1 was excluded for not specifying the diagnosis of TMD and the other because it could not be obtained in full text.^{45,46} Finally, 8 studies were included in this systematic review. The sequence and complete search are detailed in the PRISMA flow chart (Fig. 1). All 8 studies were RCTs.^{20,47–53} Table 1 shows the characteristics of the included studies.

Table 1. Evidence for the effects of botulinum toxin (BTX-A) in patients with myofascial pain related to temporomandibular disorder (TMD)

Author (year)	Study design	Sample and features	Muscle evaluated	Concentration of BTX-A	Dosage and administration	Follow-up	Relevant findings
Ernberg et al. (2011)	randomized clinical trial	21 patients Control group: isotonic saline solution	masseter	100 U of BTX-A in 1.0 mL of saline solution maximum: 100 U per patient and 50 U in the masseter muscle Control group: 1.0 mL of saline solution	1 dose, 3 administration points on each muscle Control group: 1 dose	1 week before, 1 month after and 3 months after	No significant differences in pain reduction were found between BTX-A injection and saline injection in patients with persistent myofascial pain.
Guarda-Nardini et al. (2012)	randomized clinical trial	30 patients: 22 females and 3 males (range: 20–71 years) Control group: fascial manipulation technique	temporal masseter	150 U of BTX-A for each side	1 dose of BTX-A, multiple administration points on muscle Control group: multiple 50 min sessions, 150 min in total	treatment initiation, 1 h after and 3 months after	Both treatments are equally effective in reducing pain in a follow-up of up to 3 months. The increase in mandibular range of motion was slightly bigger after BTX-A injections.
De Carli et al. (2016)	randomized clinical trial	15 patients: 13 females and 2 males (mean age: 38 years) BTX-A group (n = 7); Control group: low-level laser therapy (n = 8)	temporal masseter	500 mL of BTX-A in 1.1 mL of 0.9% saline solution 30 U in the first session 15 U in the second session	2 doses, 2 administration points in the masseter muscle and 1 administration point in the temporalis muscle	before and after the treatment	Both treatments were effective and there was no difference between them regarding pain reduction. The low-level laser effect was faster (12 days) than BTX-A (30 days).
Kürtük et al. (2019)	randomized prospective study	40 patients: 29 women and 11 men (mean age: 33.8; range: 20–60 years) Study group (n = 20); Control group: dry needling technique (n = 20)	temporal pterygoid	500 mL of BTX-A 10 cc 0.9% NaCl 25 UA 150 U per patient 25 (0.5 cc) at each trigger point	Study group: 1 dose; Dry needling group: the trigger point is injected from 8 to 10 times	treatment initiation and 6 weeks after the treatment	Pain relief at rest was more effective with the use of dry needling technique after 6 weeks. Both treatments produced significant pain relief and improved function in patients with myofascial pain.
Kurtoglu et al. (2008)	randomized prospective study	24 patients Study group (n = 12; mean age: 29.6 years; range: 16–53 years); 10 females and 2 males; Placebo group (n = 12; mean age: 23.4 years; range: 20–34 years); 10 females and 2 males	temporal masseter	100 U of BTX-A Study group: BTX-A with 2 cc saline solution; Placebo group: 2 cc saline solution	1 dose, 3 administration points in the masseter muscle and 2 administration points in the temporalis muscle (10 U each)	beginning of the study, 14 days after and 28 days after	Pain relief and improvement of psychological state after the BTX-A injections and until the 28th day.
Montes-Carmona et al. (2020)	randomized clinical trial	60 patients (range: 18–75 years) BTA group (n = 20); Lidocaine group (n = 20); Placebo group: saline solution (n = 20)	temporal masseter pterygoid	BTA group: 100–150 U 50 U of BTA in 1.25 mL of saline solution to obtain 4 units of BTA per 0.1 mL of the injection liquid; Lidocaine group: 2% lidocaine with vasoconstrictor; Saline solution group: 0.9% saline solution	1 dose in all groups, 3 administration points in the temporalis muscle, 3 administration points in the masseter muscle, 1 administration point in the lateral pterygoid muscle	before the treatment and 7, 14, 28, 60, 90, and 180 days after the treatment	BTX-A significantly reduced pain compared to saline and lidocaine. The effects lasted up to 6 months and were more intense in patients with localized myofascial pain than in patients with referred remote pain.
De la Torre et al. (2020)	randomized clinical trial	100 female patients (mean age: 36.8 ±5.6 years) Low BoNT-A group (n = 20); Medium BoNT-A group (n = 20); High BoNT-A group (n = 20); Positive control group: oral administration (n = 20); Negative control group: physiological serum (n = 20)	temporal masseter anterior	BoNT-A group: 100 U of botox diluted in different concentrations with 0.9% sterile saline solution; Saline solution group: 0.9% sterile saline solution	1 dose of 1 mL in all BoNT-A and saline groups, 5 administration points in each muscle. Oral administration group: use during the night	before the treatment and 7, 14, 21, 28, 90, and 180 days after the treatment	BoNT-A (Regardless of dose) was more effective in the reduction of persistent myofascial pain than saline solution and as effective as the occlusal appliance after 14 days and up to 6 months of follow-up.
Gupta et al. (2016)	randomized clinical trial	24 patients (range: 20–50 years) BTX-A group (n = 12); Control group: placebo (n = 12)	temporal masseter anterior	10 U of BTX-A at 10 points 30 MU for the masseter muscle; 20 MU for the anterior temporal muscle Control group: isotonic saline solution	1 dose, 3 administration points in each masseter muscle, 2 administration points in each temporalis muscle.	before the treatment, 14 days after and 28 days after the treatment	Statistically significant reduction of pain and improvement in function after the administration of BTX-A compared to the placebo that did not present a significant change in a follow-up of up to 8 months.

BTX-A – botulinum toxin type A; BoNT-A – botulinum neurotoxin type A.

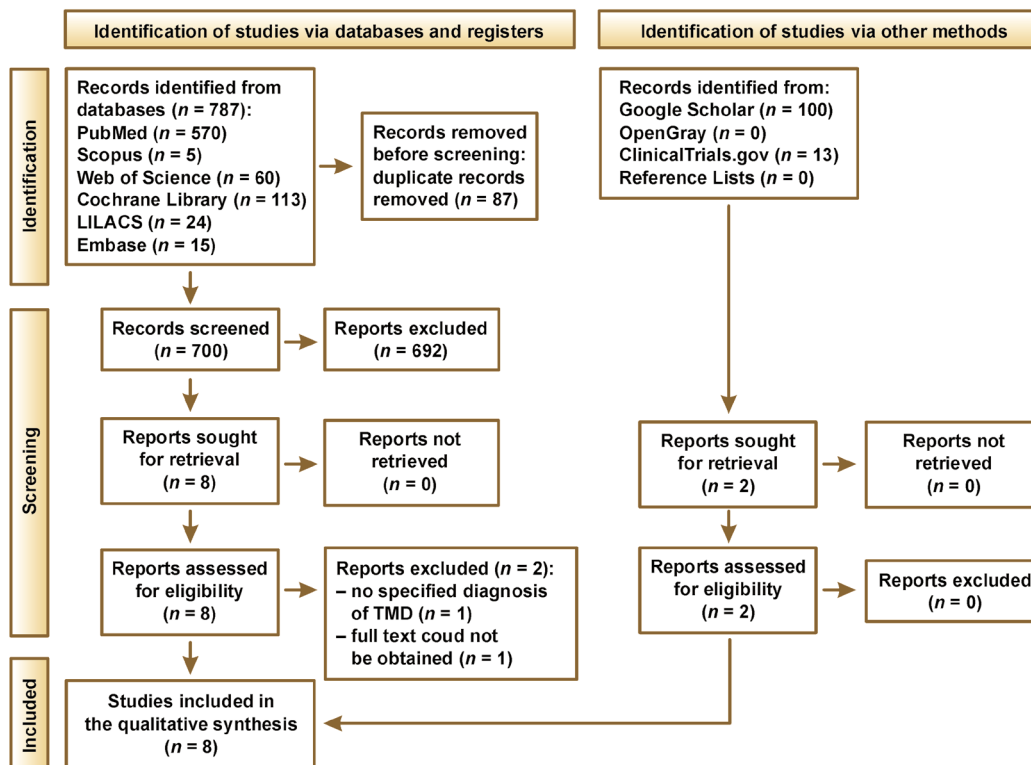


Fig. 1. PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flow chart

Study characteristics

A sample size of 314 patients were included in the analysis, the subjects being adults between 18 and 75 years of age, predominantly female, diagnosed with TMD-related myofascial pain, without complete relief of symptoms after receiving conventional treatment for at least 6 months. The patients were treated with BTX-A in concentrations ranging from 100 U to 150 U diluted in 0.1 mL to 1.1 mL of sterile saline solution, injected into different muscles. In 6 out of the 8 studies, BTX-A was injected into the masseter and temporal muscles, whereas in only 2 studies BTX-A was injected into the masseter, temporal and pterygoid muscles. Each patient received a maximum of 25–150 U of BTX-A, with an injection of 5–50 U of BTX-A at 1, 2 or 3 sites.

Risk of bias within the study

The risk of bias assessment of the 8 included RCTs^{20,47–53} was carried out using the Cochrane RoB 2.0 tool.⁴³ Out of the 8 studies, only one had a low risk of bias,⁵⁰ while in the remaining 7 studies some concerns have been raised, regarding the selection of the reported outcome in the studies describing multiple outcome measures (scales and time points, among others). However, not all data of the results obtained was shown in detail, evidencing a lack of information on the results.^{20,47–49,51–53} The evaluation of the studies is shown in Table 2 and Fig. 2.

Table 2. Assessment of risk of bias in randomized clinical trials using Cochrane tool: RoB 2.0

Author (year)	Bias arising from the randomization process	Bias due to deviations from intended intervention	Bias due to missing data	Bias in measurement of outcomes	Bias in the selection of the reported result	Overall
Ernberg et al. (2011)	low risk	low risk	low risk	low risk	some concerns	some concerns
Guarda-Nardini et al. (2012)	some concerns	low risk	low risk	low risk	some concerns	some concerns
De Carli et al. (2016)	low risk	low risk	low risk	some concerns	some concerns	some concerns
Kütük et al. (2019)	low risk	some concerns	low risk	low risk	some concerns	some concerns
Kurtoglu et al. (2008)	low risk	low risk	low risk	low risk	some concerns	some concerns
Montes-Carmona et al. (2020)	low risk	some concerns	low risk	some concerns	some concerns	some concerns
De la Torre et al. (2020)	low risk	low risk	low risk	low risk	low risk	low risk
Gupta et al. (2016)	some concerns	low risk	low risk	low risk	some concerns	some concerns

	Bias arising from the randomization process	Bias due to deviations from intended intervention	Bias due to missing data	Bias in measurement of outcomes	Bias in the election of the reported result	Overall
Ernberg et al. 2011	+	+	+	+	?	?
Guarda-Nardini et al. 2012	?	+	+	+	?	?
De Carli et al. 2016	+	+	+	?	?	?
Kütük et al. 2019	+	?	+	+	?	?
Kurtoglu et al. 2008	+	+	+	+	?	?
Montes-Carmona et al. 2020	+	?	+	?	?	?
De la Torre Canales et al. 2020	+	+	+	+	+	+
Gupta et al. 2016	?	+	+	+	?	?

Fig. 2. Evaluation of the included studies in terms of risk of bias

Results of individual studies

All the studies included in the present systematic review evaluated changes in the range of pain intensity. Three studies compared BTX-A (study group) with saline injections (placebo group),^{47,51,53} one study compared BTX-A (study group) with saline injections (placebo group) and lidocaine (control group),⁴⁹ another study compared a low-level laser (study group) with BTX-A,⁴⁸ another study compared BTX-A (study group) with the technique of dry needling (control group),⁵² another compared BTX-A (study group) with the fascial manipulation technique (control group),²⁰ and only one study compared 3 different concentrations of BTX-A with physiological saline (negative control group) and with oral appliance (positive control group).⁵⁰

The study that evaluated the safety and efficacy of 3 different doses of botulinum neurotoxin type A (BoNT-A) (low dose of BoNT-A (BoNT-A-L), medium dose of BoNT-A (BoNT-A-M) and high dose of BoNT-A (BoNT-A-H)) showed a significant decrease in the intensity of subjective pain in the 3 groups, regardless of the administered dose. Furthermore, no significant differences were found between the 3 groups,⁵⁰ which shows that even at low doses, BoNT-A can be equally effective up to 6 months after the administration.^{49,50}

Four of the studies comparing BTX-A with saline showed that BTX-A injections were clinically effective in reducing pain^{47,50,51,53} and increased the pressure pain threshold more than saline.⁵⁰

Another study evaluated the efficacy of BTX-A in the treatment of refractory masticatory myofascial pain syndrome (MMPS) and classified myofascial pain as either localized (MP), non-localized, irradiated, or referred (PR). The results showed that the changes in pain intensity values were statistically significant for the BTX-A group (all patients showed pain reduction from day 0 to day 180, except for the saline and lidocaine groups). Likewise, when comparing the MP group with the PR group that received BTX-A, the pain reduction according to the VAS was greater in the MP group, decreasing from 6 to 2 points and from 6.5 to 4 points, respectively. Although a significant decrease in pain was observed, very low values were not reached in the PR group.⁴⁹

After comparing the low-level laser therapy with BTX-A injections,⁴⁸ there were no statistically significant differences between the 2 treatments with respect to pain at the 30-day follow-up. This study reported a baseline VAS of 7 points in both groups, with a decrease to 2.75 on day 12 in the laser group and to 2.86 in the BTX-A group on day 30, demonstrating that both treatments were statistically effective. However, the effects of the low-level laser therapy were faster compared to BTX-A injections (the reduction observed at day 12 vs. day 30, respectively). These results should be taken into account in future studies.⁴⁸

Another treatment compared to BTX-A injection was the dry needling technique,⁵² in which the effectiveness on myofascial pain during chewing and rest was evaluated. Both treatments achieved a significant improvement in VAS scores during the 6 weeks of follow-up. However, with respect to myofascial pain at rest, the relief was greater with the dry needling technique. One of the 8 included studies compared BTX-A with the fascial manipulation technique.²⁰ Both treatment protocols significantly improved and decreased the intensity of myofascial pain, with no relevant clinical differences between the 2 protocols during the 3-month follow-up.

When BoNT-A (regardless of dose) was compared with the oral device, the study showed that both treatments were equally effective in treating persistent myofascial pain during the 24-week follow-up and there was no statistical difference until the last period of follow-up. However, the author also reported a reduction in muscle activity and a decrease in muscle thickness and bone volume of the condyloid and coronoid processes as dose-related adverse effects of BoNT-A (higher doses). Therefore, the BoNT-A in patients with myofascial pain should ideally be administered at low doses.⁵⁰

A meta-analysis could not be performed due to the heterogeneity of the results (differences in botulinum toxin injection protocols, sample sizes, doses, and follow-up periods).

The assessment of the level of evidence using GRADE approach showed that the 8 included RCTs^{20,47-53} presented moderate- to low-certainty evidence. A detailed description is shown in Table 3.

Table 3. Classification of the level of certainty of the evidence: The Grading of Recommendations Assessment, Development and Evaluation (GRADE) system

Comparison	Assessment of certainty of the evidence							Summary of findings	Certainty
	Number of studies (patients)	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias		
BTX-A compared to saline injections	3 (69)	RCTs	* serious	not serious	not serious	not serious	not suspected	BTX-A had a clinically significant effect on reducing pain in the 1- to 8-month follow-up compared to the saline group that did not present a statistically significant reduction.	⊕⊕⊕⊕ MODERATE
BTX-A compared to saline and lidocaine injections	1 (60)	RCT	* serious	not serious	not serious	• serious	not suspected	BTX-A significantly reduced pain compared to saline and lidocaine. The effects lasted up to 6 months and were more intense in patients with localized myofascial pain than in those with referred remote pain.	⊕⊕⊕⊕ LOW
BTX-A compared to low-level laser therapy	1 (15)	RCT	* serious	not serious	not serious	• serious	not suspected	Both treatments were effective and no difference between both treatments with respect to pain reduction was observed 30 days after starting the treatment.	⊕⊕⊕⊕ LOW
BTX-A compared to dry needling technique	1 (40)	RCT	* serious	not serious	not serious	• serious	not suspected	Both treatments showed significant pain relief at the 6-week follow-up.	⊕⊕⊕⊕ LOW
BTX-A compared to facial manipulation technique	1 (30)	RCT	* serious	not serious	not serious	• serious	not suspected	Both treatments were effective in reducing myofascial pain for up to 3 months of follow-up.	⊕⊕⊕⊕ LOW
BTX-A (3 different concentrations) compared with saline and oral apparatus	1 (100)	RCT	not serious	not serious	not serious	• serious	not suspected	BTX-A (regardless of dose) was more effective in reduction of persistent myofascial pain than physiological serum; BTX-A was also equally effective as occlusal appliance at 14 days and up to 6 months of follow-up.	⊕⊕⊕⊕ MODERATE

RCT – randomized clinical trial; BTX-A – botulinum toxin type A. Reasons for evaluation: * The evidence was downgraded by one level due to some limitations; • The evidence was downgraded by one level because the results were derived from a single study and few participants.

Discussion

Currently, there is no consensus on the most appropriate treatment protocol for myofascial pain. A multidisciplinary approach and first-line treatment are recommended, beginning with conventional therapy. However, some patients do not achieve complete pain relief and are diagnosed with refractory myofascial pain. Intramuscular injections with BTX-A have been proposed in the literature as an alternative treatment for these cases, since this neurotoxin induces a mechanism of action on the neuromuscular junction, inhibiting acetylcholine exocytosis from the nerve end plates and causing the relaxation of muscle contraction and pain relief.³⁵ Despite this, to date, the efficacy of this treatment is not very clear and therefore, the objective of this systematic review was to synthesize the current information on the effects of botulinum toxin in patients with myofascial pain related to TMDs.

In the present systematic review, BTX-A injections proved to be significantly effective in reducing the intensity of myofascial pain,^{20,47–53} regardless of the dose used (high, medium, low).⁵⁰ This is evidenced in studies comparing BTX-A with placebo,^{47,49–51,53} which demonstrated a reduction in pain clinically more efficient with BTX-A compared to placebo.^{49,51} However, 2 studies found that the difference between the 2 treatments was not statistically significant.^{47,53}

The fascial manipulation technique has been shown to be more effective in the immediate relief of self-reported pain compared to the BTX-A treatment.²⁰ However, the difference between the 2 treatment protocols was not clinically significant at 3-month follow-up, and both treatments were found to be equally effective in reducing pain.²⁰ This difference could be due to the multiple sessions (3 ± 1) that patients received compared to the BTX-A treatment that was only performed in a single session. The relaxing and calming effect that the operator transmits by exerting deep digital pressure with the fingertips or elbows on the muscle areas (establishing a positive relationship) during the 50-min sessions could also have had a psychological influence, compared to the BTX-A treatment, which has a cumulative effect.²⁰ It should be noted that the evaluation of the immediate effect of the BTX-A cannot be compared with other therapies. Such evaluation should be performed days later when the effects appear, that is, its results should be compared in the medium and long term due to the cumulative effect of the BTX-A treatment. Similar results were obtained when the low-level laser therapy was compared to the BTX-A treatment.

From the beginning to the last follow-up of the study, lidocaine injections were not significantly effective in the treatment of myofascial pain.⁴⁹ In contrast, both the dry needling technique and the administration of BTX-A showed favorable results in the relief of myofascial pain during chewing and at rest. Furthermore, the relief of myofascial pain at rest was statistically significant with the dry needling technique.⁵² Also, in a recently published

study, De La Torre Canales et al. compared acupuncture therapy with BoNT-A and saline administration. The researchers found that all 3 therapies significantly reduced self-perceived pain after 1 month of follow-up. However, there was no difference between acupuncture and BoNT-A. Both therapies were effective and superior to saline.⁵⁴

Due to its non-invasive and reversible characteristics, the oral appliance is probably the most widely used therapy aimed at reducing the symptoms of myofascial pain.⁵⁰ In our review, the oral appliance was equally effective as BoNT-A therapy. The authors of the study found no significant differences between the 2 treatments.⁵⁰ This could be due to the fact that the effect of both treatments is observed days after the start of the treatment, that is, it has a cumulative effect. Therefore, we can confirm that BTX-A compared with conventional treatments (oral appliances, fascial manipulation technique, low-level laser therapy, and dry needling technique) presents similar results. However, despite the aforementioned evidence, a definitive result cannot be established since the summary of certainty of the evidence using GRADE approach showed moderate to low certainty in the evidence of the studies (see Table 3). Moreover, the studies had limitations such as small sample sizes and short follow-up periods.

Therefore, more RCTs with larger sample sizes, longer follow-ups and the inclusion of several control groups (occlusal splints, drug therapy, acupuncture, transcutaneous electrical nerve stimulation (TENS), among others) are needed to determine the effectiveness of BTX-A in the long-term treatment of myofascial pain in patients with TMD.

Conclusions

Based on the analyzed studies, botulinum toxin appears to be as effective in controlling myofascial pain related to TMDs as conventional treatments (oral appliance, lidocaine injections, low-level laser therapy, dry needling technique, saline injections, and fascial manipulation technique).

Botulinum toxin is a useful clinical alternative adjunct to existing conservative treatments of refractory myofascial pain related to TMD.

For the control of refractory myofascial pain related to TMDs, botulinum toxin should be administered in low doses in order to avoid adverse effects related to the high-dose administration.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

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Effectiveness of the photobiomodulation therapy using low-level laser around dental implants: A systematic review and meta-analysis

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Abstract

Background. The photobiomodulation (PBM) therapy has been applied in various fields. Its use in implant dentistry has been proven through various animal, in vitro, and recently also clinical studies. However, the cumulative data of its effect around dental implants in patients is limited.

Objectives. The purpose of the present study was to evaluate whether or not the PBM therapy has a positive effect around dental implants and on implant stability.

Material and methods. The studies included in the review and meta-analysis were selected according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and the PICOS criteria. The RoB 2 tool was used for assessing the risk of bias and the RevMan software, v. 5.0, was used for meta-analysis. Quantitative analysis was done considering the implant stability measurement as the outcome. The mean and standard deviation ($M \pm SD$) values for implant stability as well as the sample size were extracted from the articles, and the inverse variance method with random effects was used for meta-analysis. The forest plots for all time intervals were inspected to estimate the heterogeneity by assessing the I^2 statistic.

Results. A total of 148 articles were initially retrieved, out of which 81 remained after duplicate removal. Ten articles were included in the review after rejecting 68 on the basis of title and abstract. Seven were eligible for quantitative analysis. The meta-analysis showed non-significant differences in primary stability in control and laser groups at baseline ($p = 0.63$) and 3 months and above ($p = 0.06$). At 2 weeks, 1 month and 2 months, the results were statistically significant with $p = 0.01$, $p = 0.02$ and $p = 0.04$, respectively.

Conclusions. The PBM therapy showed a positive effect on implant stability during the early stages of healing and can be considered for patients with dental implants.

Keywords: photobiomodulation, implant stability, low-level laser, implant dentistry

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Introduction

The most important factor to be considered in implant dentistry is the biologic union of the implant with the surrounding bone, which is termed osseointegration.¹ The cascade of phenomena that occur during osseointegration includes the formation of a blood clot, angiogenesis, the apposition of bone, and finally the remodeling of the woven bone in the peri-implant area.² Adequate implant stability plays a critical role in osseointegration. Both primary and secondary implant stability influences successful implant treatment, the former occurring due to the mechanical engagement of the implant into the bone, and the latter due to the remodeling and regeneration process during the healing phase.³ Various techniques have been advocated to improve implant stability as well as soft tissue healing around the dental implant, which in turn contributes to implant success.^{4,5}

Photobiomodulation (PBM) is a non-invasive, non-thermal therapy which through the use of low-level laser (LLL) stimulates cells to generate more energy and undergo self-repair. It involves the application of monochromatic light with a low energy density, which induces non-thermal photochemical effects on the cellular level.⁶ Photobiomodulation using low-level laser therapy (LLLT) with a low energy density stimulates the mitochondrial and cellular membrane photoreceptors to synthesize adenosine triphosphate (ATP), which in turn enhances the cell proliferation rate.⁷ It also increases the proliferation and differentiation of osteoblasts, thus having a bio-stimulatory effect on bone tissue.⁸ This potential role of PBM has been studied in the field of implant dentistry to improve implant stability, and overall hard and soft tissue healing around dental implants. Several *in vitro* and animal studies have suggested a positive effect of LLLT around the implant.^{9–14} However, considering human studies in the recent past, the effectiveness of the PBM therapy around dental implants has not been clearly shown. This systematic review and meta-analysis was conducted on the basis of clinical studies to evaluate the qualitative and quantitative effect of PBM on implant stability.

Material and methods

The systematic review was conducted in accordance with the guidelines provided in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.¹⁵ The review protocol was registered under PROSPERO International Prospective Register of Systematic Reviews (CRD42021249393). The review question was specifically constructed following the PICOS criteria:

- Population – male or female patients requiring the replacement of one or more missing teeth with dental implants;
- Intervention – the PBM therapy around dental implants;
- Comparison – patients who did not receive PBM or any other adjunctive therapy around dental implants;

- Outcome – the effect of the PBM therapy on implant stability; and
- Study design – randomized controlled trials (RCTs), clinical trials.

The review question was “Does the PBM therapy in patients receiving dental implants have an effect on implant stability?”

Search strategy

The search of electronic databases (PubMed, EBSCOhost, Cochrane, and Embase) was carried out by 2 investigators individually, without consulting one another. The following terms were searched in all databases: ‘dental implant’ OR ‘dental implants’ AND ‘photobiomodulation’ OR ‘low-level laser therapy’ OR ‘low-level laser’ OR ‘LLL’ OR ‘laser therapy’ AND ‘primary stability’ OR ‘implant stability’ OR ‘dental implant stability’ OR ‘osseointegration’. Also unpublished data was searched on ClinicalTrials.gov. The electronic databases were searched for articles published till April 2021. The EndNote™ software, v. 8.0 (<https://endnote.com/downloads>), was used to remove duplicates. The same software was used for the initial screening of the articles on the basis of their title and abstract. Eligible articles fulfilling the inclusion criteria were further assessed by accessing their full text from relevant sources. This was done individually by the 2 investigators considering the inclusion and exclusion criteria. A detailed assessment of all the citations and references was done thoroughly to widen the search. Any discrepancy between the 2 investigators was resolved by a third investigator. The inter-investigator reliability was assessed using the Cohen’s kappa score.

Inclusion and exclusion criteria

Randomized controlled trials and clinical studies that evaluated the effect of PBM using LLL and a diode laser on the conventionally or immediately placed dental implants were selected for the review. The exclusion criteria were as follows: animal studies; *in vitro* studies; case reports; reviews; books; and studies that involved extensive grafting and adjunctive procedures during implant placement, systemically ill patients, or the use of orthodontic or mini implants.

Quality assessment

The risk of bias assessment for all the included studies was carried out using the RoB 2 tool.¹⁶ According to the RoB 2 tool, 5 domains were taken into consideration to evaluate each article in terms of risk of bias, namely the randomization process, deviation from the intended intervention, missing outcome data, the measurement of the outcome, and the selection of the reported results. Accordingly, the risk of bias of the particular articles was classified as low, high or unclear.

Meta-analysis

The Review Manager software (RevMan, v. 5.4; the Cochrane Collaboration, 2020) (<https://training.cochrane.org/online-learning/core-software/revman>) was used for the

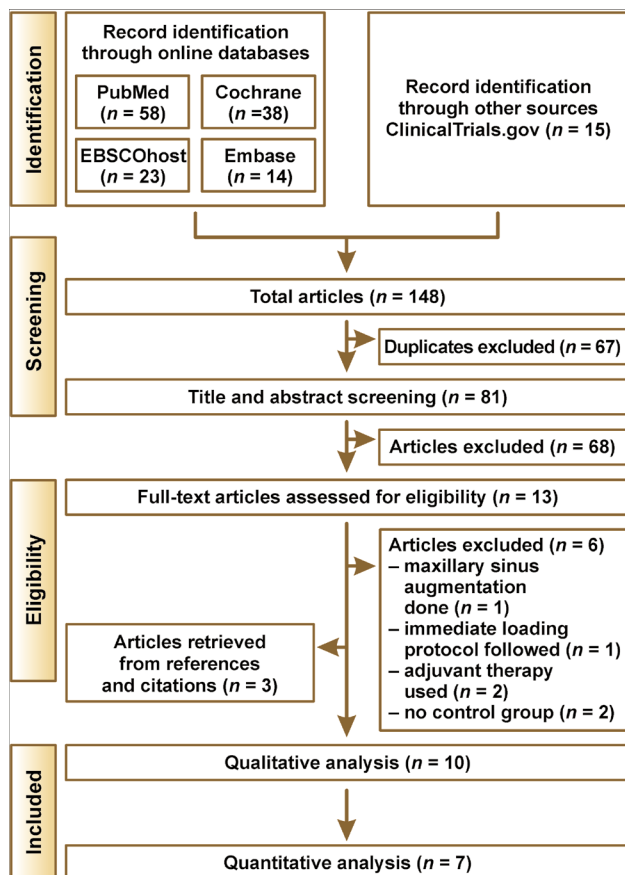


Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart of the screening process

quantitative assessment of eligible articles. The mean and standard deviation ($M \pm SD$) values for implant stability as well as the sample size were extracted from the articles, and the inverse variance method with random effects was used for meta-analysis. Implant stability measurements were taken at baseline (immediately after implant placement), 14 days (1–2 weeks), 1 month (3–4 weeks), 2 months (5–8 weeks), and 3 months or more (9–12+ weeks) after implant placement. The forest plots for all time intervals were inspected to estimate the heterogeneity by assessing the I^2 statistic, where $I^2 > 50\%$ was moderate to high heterogeneity.¹⁷

Results

The initial search identified a total of 148 relevant articles from electronic databases, out of which 81 remained after the removal of duplicates. Out of this, 68 articles were excluded based on the exclusion criteria. At the end of the study selection, 10 articles were included for qualitative^{18–27} and 7 for quantitative analysis.^{18–20,22–24,27} The study screening and the selection of articles is explained in Fig. 1. The inter-investigator reliability in the selection of articles was statistically evaluated using Cohen’s kappa analysis and a score of 0.97 was obtained.

All 10 articles raised some concerns as to the selection of the reported results (D5).^{18–27} Four studies showed a low risk of bias with regard to the randomization process (D1), deviation from the intended intervention (D2), missing outcome data (D3), and the measurement of the outcome (D4).^{18,20,24,27} The article by Memarian et al. showed an unclear risk of bias for all 5 domains (D1–D5).²¹ None of the articles in the review presented a high risk of bias (Fig. 2).

Unique ID	Study ID	Experimental group	Comparator	Outcome	Weight	D1	D2	D3	D4	D5	Overall
1	García-Morales et al.	LLLT	CONTROL	dental implant stability	1	+	+	+	+	!	!
2	Gokmenoglu et al.	LED PBM	CONTROL	dental implant stability	1	!	+	+	!	!	!
3	Mandić et al.	LLLT	CONTROL	dental implant stability	1	+	+	+	+	!	!
4	Memarian et al.	LLL and LED PBM	CONTROL	dental implant stability	1	!	!	!	!	!	!
5	Torkzaban et al.	LLLT	CONTROL	dental implant stability	1	!	+	+	!	!	!
6	Matys et al.	LASER	CONTROL	dental implant stability	1	!	+	+	!	!	!
7	Bittencourt Lobato et al.	LLLT	CONTROL	dental implant stability	1	+	+	+	+	!	!
8	Bakry et al.	LASER	CONTROL	crestal bone evaluation	1	+	+	+	!	!	!
9	Mohajerani et al.	LLLT and LED	CONTROL	dental implant stability	1	!	+	+	!	!	!
10	Gulali et al.	LLLT	CONTROL	crestal bone evaluation	1	+	+	+	+	!	!

+

low risk

!

some concerns

–

high risk

D1 randomization process

D2 deviation from the intended intervention

D3 missing outcome data

D4 measurement of the outcome

D5 selection of the reported results

Fig. 2. Quality assessment of the included studies with the use of the RoB 2 tool

LLLT – low-level laser therapy; LED – light emitting diode; PBM – photobiomodulation; LLL – low-level laser.

Characteristics of the systematic review

All the articles included in the systematic review were clinical studies, and were published between 2012 and 2020. Three of the studies had a split-mouth design,^{18,20,21} while 7 had a case–control design.^{19,22–27} The studies were conducted in Brazil,^{18,24} Turkey,¹⁹ Serbia,²⁰ Iran,^{21,22,26} Poland,²³ Egypt,²⁵ and India.²⁷ A total of 381 dental implants were initially included in the studies, out of which 194 were in the laser group, while 187 acted as control. Males and females in the age group of 20–77 years were included. In 5 studies, implant placement was done in the mandible,^{18,21,23,25,27} while the maxilla was the implant site in 3 studies^{20,22,25}; for 2 studies, the implant site was not defined.^{19,24} Primary stability evaluation was the primary outcome of all but 2 studies, in which crestal bone loss was assessed.^{25,27} The Osstell® ISQ (implant stability quotient) device was used to measure implant stability in the majority of studies.^{18–20,22,24,26} In their study, Matys et al. calculated the periotest values (PTVs) for implant stability, and then inverted their signs to adapt them to the standard ISQ values used by other studies.²³ Crevicular biochemical markers, i.e., interleukin 1 beta (IL-1 β), transforming growth factor beta (TGF- β), prostaglandin E2 (PGE2), and nitric oxide were the secondary outcomes evaluated by Gokmenoglu et al.¹⁹ Alkaline phosphatase (ALP) and early implant success were evaluated by Mandić et al.,²⁰ while IL-1 β and PGE2 were recorded by Memarian et al.²¹ Bakry et al. assessed clinical parameters, including probing depth (PD) and the distance between the implant shoulder and the peri-implant mucosa (DIM).²⁵ The evaluation period was different for all studies, and ranged from baseline to 1 year (Table 1).

The type of laser and protocol used for PBM is presented in Table 2. Diode lasers were used in all studies with a wavelength range of 626–980 nm. In their study, Memarian et al. used 2 diode lasers, one with a wavelength of 810 nm and the other with a wavelength of 626 nm, and compared the experimental groups with the control group.²¹ The time of laser application differed in the studies, ranging from immediately after implant placement to 24 days after implant placement. However, in the studies by Matys et al.²³ and Bakry et al.,²⁵ PBM was applied 1 day and 2 weeks before implant placement, respectively.

Meta-analysis

A meta-analysis was performed to evaluate the effect of the PBM therapy using LLL on implant stability. The analysis was divided into subgroups depending on the time of implant stability evaluation (Fig. 3). All the 7 studies included in the meta-analysis evaluated implant stability at baseline (immediately after implant placement), for which the result was statistically non-significant ($p = 0.63$).^{18–20,22–24,26} Implant stability evaluation at 2 weeks, 1 month and 2 months was done by all^{18–20,22,23,26}

but Bittencourt Lobato et al., who evaluated implant stability at baseline and after 3 months only.²⁴ The overall effect of the analysis calculated for all 3 subgroups was statistically significant with $p = 0.01$, $p = 0.02$ and $p = 0.04$, respectively. However, the analysis at 3 months showed statistically non-significant data ($p = 0.06$).

Heterogeneity was assessed for all time intervals. For implant stability at baseline, heterogeneity was non-significant ($p = 0.50$; $I^2 = 0\%$). Similarly, for 2 weeks, 1 month, 2 months, and 3 months there was non-significant heterogeneity with $p = 0.11$; $I^2 = 44\%$, $p = 0.16$; $I^2 = 37\%$, $p = 0.15$; $I^2 = 38\%$, and $p = 0.21$; $I^2 = 31\%$, respectively (Fig. 3).

Discussion

There have been systematic reviews and meta-analyses evaluating the effect of the PBM therapy on osseointegration, crestal bone loss, and implant stability around orthodontic or mini implants, and even evaluating PBM as surface treatment around dental implants. However, reviews assessing the efficacy of LLLT or the PBM therapy around conventional dental implants in terms of implant stability are very few.^{28–30} This review was conducted to evaluate the effect of the PBM therapy on implant stability. Various methods are used to assess implant stability, such as insertion torque values, the reverse torque test, the percussion test, ISQ, and PTVs.³¹ Among these, the most reliable are ISQ and PTVs, which were used in the studies included in the present meta-analysis.^{18–20,22–24,26}

The PBM therapy using LLL has shown positive effects in studies done on implants in animals.^{9–14} This review focused on clinical trials only. Five out of 10 studies in the review concluded that the PBM therapy had no effect around dental implants.^{18,20,22,24,25} Statistically non-significant differences were observed for implant stability as well as ALP activity around dental implants in the study by Mandić et al.²⁰ However, when evaluating the recorded outcome variables in this study, the laser group had higher values at the end of the study for both implant stability and ALP activity.²⁰ This phenomenon can be correlated with the relationship between LLLT stimulation and the tissue stem cells reacting to it, inducing the new formation and healing of the peri-implant soft tissues and the peri-implant bone.³² Similar results were found in the studies by Torkzaban et al.²² and Bittencourt Lobato et al.,²⁴ even though the observed differences were not statistically significant. The pooled result of all the studies included in the meta-analysis showed a positive effect of the PBM therapy on dental implant stability in contrast to the other systematic review results. Crestal bone loss was evaluated by 2 studies; Bakry et al. concluded that the PBM therapy had no effect,²⁵ while Gulati et al. showed a positive effect.²⁷

No homogeneity was found in the laser parameters used in the different studies included in the review. This may be one of the reasons for the discrepancy in the results of the studies.

Table 1. Characteristics of the included studies

Study	Country	Study design	Number of implants	Average age [years]	Sex		Implant site	Implant producer	Bone type	Outcome	Evaluation time	Study conclusion
					M	F						
García-Morales et al. 2012	Brazil	SM	29 LG (15) CG (14)	36	2	6	posterior mandible	Dentsply, Mannheim, Germany	–	implant stability (Osstell ISQ device)	immediately, 10 days, and 3, 6, 9, and 12 weeks postoperatively	no effect
Gokmenoglu et al. 2014	Turkey	CC	22 LG (10) CG (12)	48	9	6	–	Dentsply, Mannheim, Germany	type 2 or 3	implant stability (Osstell ISQ device) IL-1 β , TGF- β , PGE2, and nitric oxide	immediately, and 2, 4, 8, and 12 weeks postoperatively 4 and 12 weeks postoperatively	positive effect
Mandić et al. 2015	Serbia	SM	40 LG (20) CG (20)	61	6	6	posterior maxilla	Bredent, Senden, Germany	–	implant stability (Osstell ISQ device) PICF early implant success	immediately, and 1, 2, 3, 4, 5, and 6 weeks postoperatively 7, 14, 21, and 28 days postoperatively 6 weeks postoperatively	no effect
Memarian et al. 2018	Iran	SM	36 LG (12) LG (12) CG (12)	–	–	–	anterior mandible	Dio, Busan, South Korea	type 2 or 3	implant stability (PTVs) inflammatory biomarkers	at the day of surgery, and 3, 4 and 8 weeks after surgery 4 and 8 weeks postoperatively	positive effect
Torkzaban et al. 2018	Iran	CC	80 LG (40) CG (40)	42	9	10	maxilla	Dio, Busan, South Korea	D3 and D4	implant stability (Osstell ISQ device)	immediately, 10 days, and 3, 6 and 12 weeks postoperatively	no effect
Matys et al. 2019	Poland	CC	40 LG (18) CG (22)	47	16	8	posterior mandible	SuperLine Dentium, Cypress, USA	D2	implant stability (PTVs)	immediately, 2 and 4 weeks, and 2 and 3 months postoperatively	positive effect
Bittencourt Lobato et al. 2019	Brazil	CC	46 LG (23) CG (23)	51	–	–	–	Straumann Basel, Switzerland	–	implant stability (Osstell ISQ device)	immediately and at the abutment selection phase	no effect
Bakry et al. 2020	Egypt	CC	12 LG (6) CG (6)	40	–	–	posterior maxilla	Zimmer Dental, Carlsbad, USA	–	crestal bone level (RVG) clinical parameters	immediately, and 2, 12 and 26 weeks postoperatively immediately, and 2, 12 and 26 weeks postoperatively	no effect
Mohajerani et al. 2020	Iran	CC	56 LG (28) CG (28)	38	32	26	posterior mandible	Zimmer Dental, Carlsbad, USA	–	implant stability (Osstell ISQ device)	immediately, and 10, 21, 42, and 63 days postoperatively	positive effect
Gulati et al. 2020	India	CC	20 LG (10) CG (10)	35	13	7	posterior mandible	Adin Dental, Bielsko-Biała, Poland	type 2 or 3	crestal bone level (IOPA) CBCT	immediately, 6 weeks and 6 months following prosthesis loading, and at 1 year immediately and at 1 year	positive effect

M – male; F – female; SM – split-mouth; CC – case-control; LG – laser group; CG – control group; ISQ – implant stability quotient; IL-1 β – interleukin 1 beta; TGF- β – transforming growth factor beta; PGE2 – prostaglandin E2; PICF – peri-implant crevicular fluid; PTVs – periosteal values; RVG – radiovisiography; IOPA – intraoral periapical radiograph; CBCT – cone-beam computed tomography.

Table 2. Specifications of the lasers used in the included studies

Study	Laser	Laser specifications	Dosage
García-Morales et al. 2012	GaAlAs diode LLL Thera Lase Surgery; DMC, São Carlos, Brazil	wavelength: 830 nm mode: contact mode power: 86 ± 2 mW laser spot size: 0.0028 cm ² energy density: 92.1 J/cm ² irradiation time per point: 3 s total points: 20 points (9 at the vestibular, 9 at the lingual, 1 at the distal, and 1 at the mesial region of the implant) total energy per session: 8 J	immediately, and 2, 4, 6, 8, 10, 12, and 14 days postoperatively
Gokmenoglu et al. 2014	Osseopulse AR 300; Biolux Research, Vancouver, Canada	wavelength: 626 nm mode: contact mode (extraoral) power: 185 mW laser spot size: 4.80 cm ² energy density: 46.2 J/cm ² average intensity: 38.5 mW/cm ² irradiation time: 20 min total energy per session: 222 J	immediately, and 1, 2 and 3 weeks (3 times per week) postoperatively
Mandić et al. 2015	GaAlAs laser Medicolaser 637; Technoline, Belgrade, Serbia	wavelength: 637 nm mode: non-contact mode (extraoral) power: 40 mW energy density: 6.26 J/cm ²	every 24 h for 7 days postoperatively
Memarian et al. 2018	diode laser Doctor Smile; Lambda, Brendola, Italy	wavelength: 810 nm mode: contact mode power: 50 mW laser spot size: 1 cm ² irradiation time: 400 s	immediately, and 3, 7, 10, and 14 days postoperatively
	Osseopulse AR 300; Biolux Research, Vancouver, Canada	wavelength: 626 nm mode: contact mode (extraoral) power: 185 mW laser spot size: 4.80 cm ² energy density: 46.2 J/cm ² average intensity: 38.5 mW/cm ² irradiation time: 20 min	immediately, and 3, 7, 10, and 14 days postoperatively
Torkzaban et al. 2018	Epic 10; Biolase, Irvine, USA	wavelength: 940 nm mode: contact mode power: 100 mW laser spot size: 0.2826 cm ² average energy density: 14.18 J/cm ² average power density: 354.6 mW/cm ² irradiation time per point: 40 s total points: 2 points (at the buccal and palatal sites of the implant) total energy per session: 8 J	2, 4, 6, 8, 10, and 12 days postoperatively
Matys et al. 2019	red diode laser Smart M; Lasotronix, Piaseczno, Poland	wavelength: 635 nm mode: contact mode power: 100 mW laser spot size: 0.5024 cm ² average power density: 99.04 mW/cm ² irradiation time per point: 40 s total points: 2 points (at the buccal and lingual sites of the implant) total energy per session: 8 J	1 day preoperatively, immediately, and 2, 4, 7, and 14 days postoperatively
Bittencourt Lobato et al. 2019	GaAlAs diode laser Therapy XT; DMC, Sao Carlos, Brazil	wavelength: 808 nm power: 50 mW laser spot size: 0.4 cm ² irradiation time per point: 1.23 min total points: 6 points (2 at the labial, 2 at the lingual and 2 at the occlusal site of the implant) total energy per session: 66 J	immediately after implant placement
Bakry et al. 2020	diode laser Smart M Pro; Lasotronix, Piaseczno, Poland	wavelength: 980 nm mode: contact mode power: 2 W irradiation time: 5 min frequency: 300 Hz	2 weeks before implant placement, and 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 days postoperatively
Mohajerani et al. 2020	–	wavelength: 830 nm (laser) + 632 nm (LED) power: 10 mW laser spot size: 0.0015 cm ² irradiation time: 20 min	every day for 10 days postoperatively
Gulati et al. 2020	Photon Plus; Zolar Technology & Mfg Co. Inc., Mississauga, Canada	wavelength: 980 nm power: 0.1 W laser spot size: 0.25 cm ² energy density: 4 J/cm ² irradiation time per point: 10 s total points: 6 points (at the mesiobuccal, distobuccal, midbuccal, midlingual, mesial, and distal areas) total energy per session: 6 J	immediately following osteotomy and prior to implant placement, and 3, 7 and 14 days postoperatively

GaAlAs – gallium aluminum arsenide.

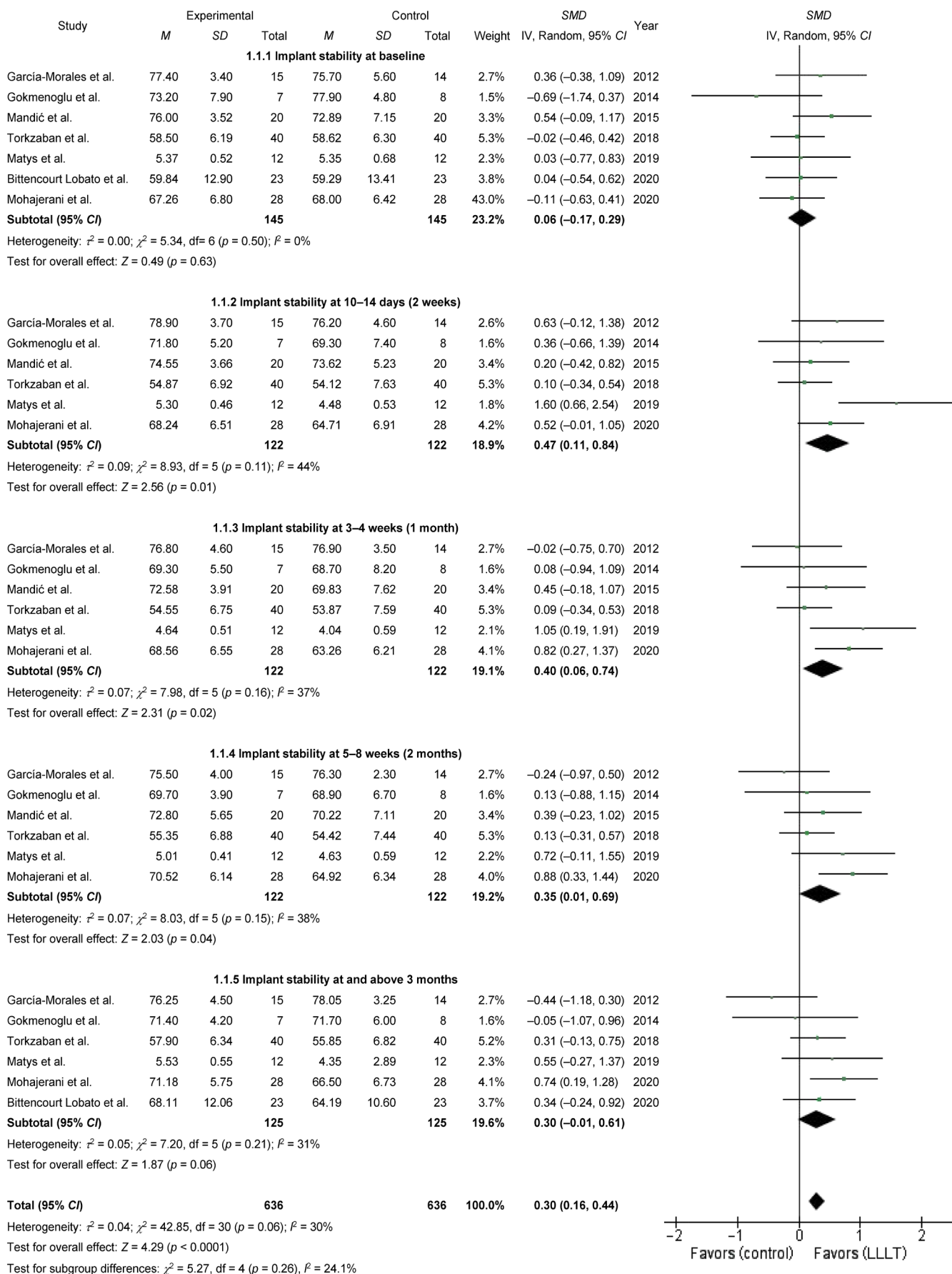


Fig. 3. Forest plots of dental implant stability for experimental (laser) and control groups at baseline, 2 weeks, 1 month, 2 months, and 3 months and above M – mean; SD – standard deviation; SMD – standardized mean difference; CI – confidence interval; df – degrees of freedom.

A laser of a higher wavelength benefits, as it penetrates deeper into the tissues and has a positive osteogenic effect.³³ Considering all the studies included in this review and their results, a standardized protocol for the PBM therapy using LLL around dental implants must be implemented.

Limitations

The current review consisted of 10 articles which were eligible for qualitative analysis, and 7 out of them were selected for quantitative analysis. This number is relatively small for a review, which is due to the fact that the topic is new and still under modification. Future research in this field should be considered for a review in the future.

Conclusions

The PBM therapy using LLL shows a positive effect on implant stability at different intervals, especially during the early stages of healing, and is worth considering for patients with dental implants. The need of the hour is to develop and test a standardized laser protocol for the PBM therapy to improve tissue around dental implants. With laser becoming one of the most popular modalities in clinical dentistry and being available to many clinical setups now, its use to enhance implant stability and healing can be of great benefit in implant dentistry.

Ethics approval and consent to participate

Not applicable.

Data availability


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


Consent for publication

Not applicable.

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Influence of the anesthetic modality on the development of neurological injury after lower third molar extraction: A systematic review of the literature

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

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Abstract

The aim of this study is to evaluate if the risk of neurological injury to the inferior alveolar nerve (IAN) and the lingual nerve (LN), following the extraction of lower third molars are influenced by the anesthetic modality (local anesthesia (LA) vs. general anesthesia (GA)).

A systematic search was performed through the PubMed, Scopus, Cochrane Library, an Web of Science databases; furthermore, a manual search was performed by analyzing the references of full-text articles.

From a total of 309 studies (collected after the removal of duplicates), 6 studies were selected. Of these, 4 reported a correlation between GA and nerve damage, while the other 2 did not show an obvious association. The level of bias in the studies was also calculated. Only 2 studies showed a medium risk of bias, while 4 studies showed a high risk of bias; no study showed a low risk of bias. Four of the 6 studies highlighted a higher incidence of IAN and LN injury, following the extractions performed under GA.

Although no scientific evidence is yet available, due to the scarcity and the limited quality of the studies in the literature, considering the risk–benefit ratio, LA should be the first choice in lower third molar surgery.

Keywords: extraction, inferior alveolar nerve, anesthesia, neurological injury, lower third molars

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Introduction

The lower third molar is situated between the second lower molar and the mandibular ramus.

The primary reasons for the impaction of the wisdom tooth are to be researched in the lack of space, malposition, unfavorable eruption angulation, or physical impediments along the pathway of eruption.¹ The etiology of tooth impaction may be associated with abnormalities in tooth development, and it is related to inherent genetic components and specific environmental conditions.²

The impaction of mandibular third molars is a condition associated with a different degree of difficulty during surgery and a higher risk of complications. The mesio-angular impaction of third molars is the most frequent situation.³ Most common complications occur when the surgical removal is performed with the altered position of the wisdom tooth, which is generally more difficult, and in the elderly patient.^{3,4}

Complications are usually local, like a hematoma or infections, and temporary, like iatrogenic inferior alveolar nerve (IAN) or lingual nerve (LN) injury.^{5,6} For this reason, the 1979 conference of the American National Institute of Health (NIH) suggested that third molars should be removed when there is evidence of pathological changes or irreversible pathology.⁷

In ethical terms, it is not advisable to perform a surgical procedure that carries a morbidity risk without valid indications. There are many indications for the extraction of the impacted lower third molar that are derived from the clinical symptomatology with the distinction between 'symptomatic' and 'asymptomatic' teeth. However, the term 'asymptomatic' is ambiguous, since the lack of symptoms should not be confused with the lack of pathology. Some diseases could remain asymptomatic before being diagnosed despite the presence of pathological (clinical or radiographic) signs. It is also important to consider clinical situations where no pathology has been developed yet, but predisposing factors are present, such as plaque accumulation, common in the case of partially erupted molars or teeth affected by dysodontiasis.

Intraoperative pain control is an intrinsic part of a surgical procedure. In most cases, third molar surgery can be performed under local anesthesia (LA), although in particular cases, general anesthesia (GA) is suggested. General anesthesia should be limited to those patients and clinical situations in which LA cannot be used: uncooperative patients and dental phobia; allergy to local anesthetics; acute and extended infections; and extensive dental or maxillofacial surgery. In the literature, there are still no precise indications as to the choice of the anesthetic modality with regard to third molar surgery. On the other hand, considering that many types of risk, e.g., rare and unpredictable death, are associated with GA, this anesthetic choice should be taken into account only if necessary.⁸

Therefore, the aim of this systematic review was to assess the influence of various modalities of anesthesia, LA

vs. GA, used for the extraction of the lower third molar on neurological injury to IAN and/or LN.

Material and methods

The present systematic review followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement⁹ and used a previous systematic review as a template.¹⁰

The following PICOS (Population, Intervention, Comparison, Outcome, and Study design) criteria were set:

- **Participants:** patients undergoing lower third molar surgery;
- **Intervention:** lower third molar extraction under LA;
- **Comparison:** patients whose surgery had been performed under GA;
- **Outcome:** neurologic injury to IAN or LN; and
- **Study design:** systematic review.

Search strategy

The review was realized with the use of scientific databases: PubMed; Scopus; Cochrane Library; and the Web of Science, from the inception to the latest research in August 2019. A manual search was also performed among the references of all full-text articles. No language restriction was applied.

The search on Pub Med was performed using the following algorithm: (local OR general) AND (anaesthesia OR anesthesia) AND (third OR 3rd OR lower OR mandibular OR wisdom OR impacted) AND (molar OR molars OR tooth OR teeth) AND (extraction OR extractions OR removal OR removals) AND (injury OR injuries OR damage OR lesion OR lesions OR disturbance OR disturbances) AND (lingual nerve OR mandibular nerve OR inferior alveolar nerve). The search strategy applied in Scopus involved the same Boolean string, including the article title, abstract and keywords. In the case of the Cochrane Library search, the "Advanced search" tool was used, choosing all content types. For the Web of Sciences, the "Advanced search" was used, selecting all languages and all document types.

Study selection

The selected studies had to meet the pre-defined eligibility criteria. They had to be randomized clinical trials (RCTs), controlled clinical trials (CCTs), or prospective cohort studies (PCs) or retrospective studies (RSs), with or without a control group.

Studies that considered 2 groups of patients undergoing lower third molar extraction were included, and studies in which 3 groups were considered because of the addition of the LA plus sedation group were also taken into account.

Studies that investigated the risk of developing nerve injury (of IAN and LN) in a group of patients treated under GA, in a group of patients treated under LA and in a group of pa-

tients treated under LA plus sedation (if the 3rd type of anesthetic modality was taken in consideration) were collected.

Studies that distinguished temporary and permanent injury after the period of follow-up, with at least 6 months of follow-up were included.

Case report, case series, studies enrolling less than 10 subjects, comments, expert opinions, letters to the editor, reviews, and studies that analyzed the same sample as a pre-existent study were excluded.

Studies that did not evaluate the anesthetic modality as a parameter in the development of neurologic injury and studies that evaluated a single type of anesthetic modality (only a group of patients treated under LA or GA) with regard to nerve injury after lower third molar extraction were excluded.

Studies that did not distinguish temporary and permanent injury after the period of follow-up were excluded.

Redundant studies were excluded.

Data items

The following data was collected: study design; anesthetic modality; (LA, GA and LA plus sedation); sample size; gender and age; number of teeth removed; operators' experience; surgical difficulty rated according to different classifications (Winter's classification, Wharfe's score, and

Pell and Gregory's classification); use of a lingual retractor (if used, specifying the type); follow-up; nerve injury classified as temporary or permanent (persisting after 6 months); and clinical implications according to the authors.

Assessment of the risk of bias in individual studies

The risk of bias in individual studies was evaluated according to a modified Downs and Black tool.¹¹ The original Downs and Black tool¹² consists in the calculation performed by evaluating each study across 5 domains, including:

- Reporting (10 items);
- External validity (3 items);
- Internal validity – bias (7 items)
- Internal validity – confounding (6 items); and
- Power (1 item).

The maximum possible score is 32. In the current review, certain adaptations were introduced to adhere to the studies dealing with the topic “nerve injury after third molar removal under local or general anesthesia”.

These were as follows (Table 1):

- Item 19 (Was compliance with the intervention/s reliable?) was not considered, as in the present research, the compliance of the patients was not required to evaluate the results;

Table 1. Modified Downs and Black tool used for the analysis of the risk of bias of non-randomized clinical trials (judgments and scores for each item as follows: No/Not applicable (0); Yes (1))

Domain	Question
Reporting	1. Is the objective of the study clearly described?
	2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?
	3. Are the characteristics of the patients included in the study clearly described?
	4. Are the interventions of interest clearly described?
	5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?
	6. Are the main findings of the study clearly described?
	7. Does the study provide the estimates of random variability in the data for the main outcomes?
	8. Have all important adverse events that may be a consequence of the intervention been reported?
	9. Have the characteristics of the patients lost to follow-up been described?
	10. Have actual probability values been reported (e.g., 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?
External validity	11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?
	12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?
	13. Were the staff, places and facilities where the patients were treated representative of the treatment the majority of patients receive?
Internal validity – bias	14. Was an attempt made to blind the study subjects to the intervention they received?
	15. Was an attempt made to blind those measuring the main outcomes of the intervention?
	16. If any of the results of the study were based on 'data dredging', was this made clear?
	17. In trials and cohort studies, were the analyses adjusted for different lengths of the follow-up of the patients, or in case-control studies, was the time period between the intervention and the outcome the same for cases and controls?
	18. Were the statistical tests used to assess the main outcomes appropriate?
	19. Were the main outcome measures used accurate (valid and reliable)?
Internal validity – confounding	20. Were the patients in different intervention groups (trials and cohort studies) or were cases and controls (case-control studies) recruited from the same population?
	21. Were the patients in different intervention groups (trials and cohort studies) or were cases and controls (case-control studies) recruited over the same period of time?
	22. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?
	23. Was the loss of patients to follow-up taken into account?
Power	24. Sample size calculation

- Item 23 (Were study subjects randomized to intervention groups?) and Item 24 (Was the randomized intervention assignment concealed from both patients and healthcare staff until recruitment was complete and irrevocable?) were not considered, as not all of the included studies were randomized; and
- Item 27 (Power) was simplified to “Sample size calculation”.

Thus, the domains of the modified tool were as follows: reporting (10 items); external validity (3 items); internal validity – bias (6 items); internal validity – confounding (4 items); and power (1 item), with a maximum score of 25. The overall risk of bias was defined as follows:

- high: total score ≤ 16 ;
- medium: total score >16 and <22 ; and
- low: total score ≥ 22 .

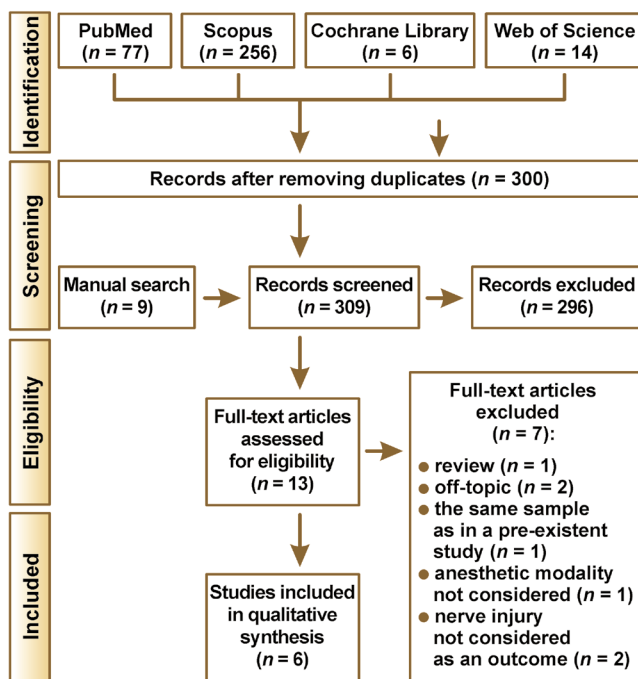


Fig. 1. Flow diagram of the search strategy

Table 2. Studies excluded after full-text consideration with the corresponding main reason for exclusion

Authors	Year	Reference	Main reason for exclusion
Nowak et al.	2014	<i>Dent Med Probl.</i> 2014;51(2):225–230	review
Renton	2013	<i>Br Dent J.</i> 2013;215(8):393–399	anesthetic modality not considered
Gülicher and Gerlach	2000	<i>Oral Maxillofac Surg.</i> 2000;4(2):99–104	the same sample as in a pre-existent study
Loescher et al.	2003	<i>Dent Update.</i> 2003;30(7):375–380,382	off-topic
Edwards et al.	1999	<i>Ann R Coll Surg Eng.</i> 1999;81(2):119–123	paresthesia not considered as an outcome
Edwards et al.	1998	<i>Br J Oral Maxillofac Surg.</i> 1998;36(5):333–340	paresthesia not considered as an outcome
Worrall et al.	1998	<i>Br J Oral Maxillofac Surg.</i> 1998;36(1):14–18	off-topic

Results

Study search

The results of the automatic and manual search are summarized in Fig 1. From a total of 309 articles retrieved, 7 of the 13 full-text articles assessed for eligibility were excluded for the reasons provided in Fig. 1 and Table 2.

Finally, 6 studies were judged eligible according to the inclusion/exclusion criteria (Table 3).

Study design

The 6 selected studies included 1 retrospective observational study (ROS),⁸ 3 prospective studies (PSs),^{13–15} 1 prospective longitudinal study (PLS),¹⁶ and 1 RS.¹⁷ In half of the selected studies, the extractions were performed under LA or GA^{8,14,17}; in the remaining half, the extractions were performed under LA, under GA or under LA plus sedation.^{13,15,16} The sample size in the selected studies ranged from 387¹³ to 6,803 subjects.¹⁷ All the studies included both males and females. In 3 cases,^{14,16,17} information on gender distribution was not available. The mean age of patients ranged between 24.92 \pm 4.67 years¹³ and 41.3 \pm 17.8 years⁸; in 2 cases, the mean age of patients was not considered.^{14,17}

The number of teeth extracted under LA was in the range between 105¹⁶ and 631,¹⁵ under GA it was between 194⁸ and 535,¹³ and the range for LA plus sedation was between 15¹⁵ and 41.¹³ Only Nguyen et al. have not specified how many teeth were extracted under LA or GA.¹⁷

In 4 cases, the extractions were performed by surgeons with different levels of experience^{14–17}; in the study by Brann et al., the operator's experience was not considered.¹³ Only in 1 study, the extractions were performed by a single surgeon with high experience.⁸

Surgical difficulty was rated according to different classifications. Three studies recorded the degree

Table 3. Summarized data of the 6 studies included in the review

Study	Type	Anesthetic modality	Sample size <i>n</i>	Gender <i>n</i> and age [years] <i>M</i> ± <i>SD</i>	Number of teeth removed	Operator's experience	Surgical difficulty	Use of a lingual retractor	Follow-up period	Number of cases of nerve injury		Clinical implications
										T	P	
Costantinides et al. ⁸ 2016	ROS	LA	340	M: 283 F: 251 41.3 ± 17.8	340	single surgeon, high experience	P&G: I-A 271 I-B 29 I-C 12 II-A 15 II-B 7 II-C 2 II-A 0 II-B 2 II-C 2	PE	6 months	1 IAN	1 IAN	GA seems to increase the risk of developing IAN and LN lesions
										0 LN	0 LN	
Brann et al. ¹³ 1999	PS	GA	271	M: 250 F: 117	535	NA	WS 8	HR/NA	6 months	5 a	5 b	GA seems to increase the risk of developing nerve injury
										194	1 LN	
Hill et al. ¹⁴ 2001	PS	GA	234	NA	430	4 surgeons, different experience	WS 5.5	HR	6 months	8 a	0	no significant difference in the adverse effects between the 2 modalities
										201	0	
Gülcher and Gerlach ¹⁵ 2001	PS	GA	NA	M: 516 F: 590	460	13 surgeons, different experience	WC: H 53 V 350 MA 490 DA 159 Trans 48 O 6	PE	6 months	c	d	a high risk of nerve injury in procedures under GA
										631	e	
Rehman et al. ¹⁶ 2002	PLS	GA	273	30.00 ± 10.58	474	different surgeons, different experience	WS 5.7	HR/HOR, 45	6 months	5 IAN	f	no significant relationship between nerve damage and anesthetic modality
										105	0 LN	
Nguyen et al. ¹⁷ 2014	RS	LA	6,803	NA	11,599	different surgeons, different experience	NA	NA	12 months	0	–	surgery under GA is a risk factor for permanent IAN injury, no factor increases the risk of LN injury
										28	8 LN	

M – mean; *SD* – standard deviation; T – temporary; P – permanent; ROS – retrospective observational study; PS – prospective study; PLS – prospective longitudinal study; RS – retrospective study; LA – local anesthesia; GA – general anesthesia; M – male; F – female; WC – Winter's classification; WS – Wharfedale score; P&G – Pell and Gregory's classification; H – horizontal; V – vertical; MA – mesioangular; DA – distoangular; Trans – transversal; O – other; PRE – Pritchard elevator; HR – Howarth retractor; PE – periosteal elevator; HOR – Hovell retractor; IAN – inferior alveolar nerve; LN – lingual nerve; a – merging cases of IAN and LN injury; b – merging cases of IAN and LN injury in the LA, GA and LA + sedation groups; c – a total of 39 cases of IAN injury by merging the LA, GA and LA + sedation groups; d – a total of 10 cases of IAN injury by merging the LA, GA and LA + sedation groups; e – a total of 4 cases of LN injury by merging the LA, GA and LA + sedation groups; f – a total of 3 cases of LN injury by merging the LA and GA groups; NA – data not available.

of surgical difficulty by means of Wharfe's score.^{13,14,16} Hill et al. found a score of 5.5 without differentiating the scores between LA and GA,¹⁴ the other 2 studies found similar Wharfe's scores for the subjects treated under LA and the subjects treated under GA.^{13,16} Winter's classification was used by Gülicher and Gerlach to find out the prevalence of the positions of the extracted teeth (horizontal (53), vertical (350), mesioangular (490), distoangular (159), transversal (48), and other (6)) without specifying the anesthetic modality.¹⁵ Costantinides et al. assessed surgical difficulty by using Pell and Gregory's classification, starting from class I-A (271 teeth in the LA group and 58 teeth in the GA group) up to class III-C (2 teeth in the LA group and 15 teeth in the GA group).⁸ In the remaining study, the preliminary staging before the extractions was not clearly described.¹⁷

A lingual retractor was used in 3 cases.^{13,14,16} In 1 case, the Howarth retractor was used in all procedures of extraction¹⁴; the Howarth retractor was also used in the study by Brann et al. for all the teeth requiring bone removal and the retraction of lingual tissues¹³; in another case, the Howarth or Hovell retractors were used based on the choice of the surgeons (in 45 procedures under LA and in 340 procedures under GA).¹⁶ The Prichard elevator was used in 1 study, when necessary, protecting but not retracting the lingual flap.⁸ Gülicher and Gerlach used a non-specified periosteal elevator to protect the lingual nerve.¹⁵ Nguyen et al. has not specified the use of a lingual retractor or a periosteal elevator.^{15,17}

The period of follow-up was 6 months.^{8,13–16} Nguyen et al. followed the patients for a period of 12 months.¹⁷

Clinical outcomes

Costantinides et al. did not observe any cases of LN injury (temporary or permanent) after LA.⁸ Only 1 case (0.29%) of temporary IAN injury was observed after a week, which persisted after the period of follow-up and developed into permanent injury. In this single case, the extracted tooth showed a canal between the roots, in which IAN was entrapped. Therefore, the nerve lesion could be ascribed to the complications related to the anatomical conformation of the extracted tooth. Nine cases (4.64%) of temporary IAN injury and 4 cases (2.06%) of temporary LN injury were observed after GA. One case (0.52%) of permanent LN injury was noticed following GA.⁸

Brann et al. found 5 cases (3.52%) of temporary nerve injury after LA, 90 cases (16.82%) after GA and 1 case (2.44%) for LA plus sedation, without distinguishing IAN and LN injury.¹³ Five cases (0.70%) of permanent nerve injury were observed without specifying the anesthetic modality and the nerve involved.¹³

In the study by Hill et al., 8 cases (3.98%) of temporary

nerve injury to IAN and LN related to LA, and 16 cases (3.72%) of temporary nerve injury to IAN and LN related to GA were detected.¹⁴ No cases of permanent nerve injury were found either for LA or for GA after the follow-up period.¹⁴

Gülicher and Gerlach found 8 cases of temporary injury to LN under LA, 1 case for LA plus sedation and 14 cases under GA.¹⁵ Four cases (0.36%) of permanent LN injury and 10 cases (0.90%) of permanent IAN injury were observed, without specifying the anesthetic modality.¹⁵

Rehman et al. found 9 cases (8.57%) of postoperative temporary nerve injury – 5 (4.76%) to IAN and 4 (3.81%) to LN – following LA, while 37 cases (7.81%) of temporary nerve injury were observed in the GA group (15 (3.16%) to IAN and 22 (4.64%) to LN).¹³ No cases of nerve injury were observed in the group of LA plus sedation. After the follow-up period, no cases of permanent injury to LN were noticed for any anesthetic modality; conversely, a total of 3 cases (0.49%) of nerve injury to IAN were observed, not specifying the anesthetic choice.¹³

In their study, performed on 11,599 cases of mandibular third molar removal (6,803 patients), Nguyen et al. found 1 case of temporary LN injury and no cases of permanent LN injury following LA; 4 cases of temporary IAN injury and 8 cases of permanent IAN injury were found for LA.¹⁷ Fifty cases of temporary nerve injury were observed – 8 to LN and 42 to IAN – after GA. Six cases of permanent LN injury and 15 cases of permanent IAN injury were detected after GA.¹⁷

Main reported results and clinical implications

In their conclusions, 3 studies suggested that mandibular third molar surgery under GA seemed to increase the risk of nerve injury as compared to surgery under LA.^{8,13,15}

Nguyen et al. in their conclusions observed an increased risk of permanent IAN injury under GA and concluded that no factor increased the risk of LN injury.¹⁷

In 2 studies, no significant relationships were found between the procedures under LA and GA and nerve injury.^{14,16}

Risk of bias in individual studies

According to the risk of bias analysis, only 2 studies were judged to have a medium risk of bias, with an overall score between 16 and 22,^{8,13} and a higher score of 19.⁸

The remaining 4 studies were judged to have a high risk of bias,^{14–17} with a lower score of 12.¹⁷ The internal validity – bias items were related to low scores, with the exception of 1 study.⁸ Full details are summarized in Table 4.

Table 4. Risk of bias in the included studies according to the modified Down and Black tool

Item	Costantinides et al. ⁸	Brann et al. ¹³	Hill et al. ¹⁴	Gülicher and Gerlach ¹⁵	Rehman et al. ¹⁶	Nguyen et al. ¹⁷
1	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)
2	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)
3	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)	no
4	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)
5	yes (2)	yes (2)	partial (1)	yes (2)	yes (2)	yes (2)
6	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)
7	no	no	no	no	no	no
8	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)
9	no	no	no	no	no	no
10	yes (1)	yes (1)	no	yes (1)	yes (1)	yes (1)
11	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)	unclear
12	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)	unclear
13	yes (1)	yes (1)	unclear	unclear	yes (1)	unclear
14	no	no	no	no	no	no
15	yes (1)	unclear	yes (1)	unclear	unclear	unclear
16	no	no	no	no	no	no
17	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)
18	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)
19	yes (1)	unclear	unclear	unclear	unclear	unclear
20	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)
21	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)	yes (1)
22	unclear	unclear	no	unclear	no	no
23	yes (1)	yes (1)	unclear	unclear	unclear	no
24	no	no	no	no	no	no
Total	19	17	14	15	16	12
Overall risk of bias	medium	medium	high	high	high	high

Discussion

The present review examined the potential development of nerve injury after lower third molar extraction performed under LA and the same intervention performed under GA. The number of retrieved studies (6 studies) is still limited, since this aspect has been poorly investigated. Moreover, the heterogeneous designs and recordings of the included studies did not allow any meta-analysis, while the direct comparisons of the obtained results are not fully applicable.

Four of the 6 studies showed an association between GA and nerve injury.^{8,13,15,17} Conversely, 2 of the included studies did not show a significant relationship between nerve damage and the anesthetic modality.^{14,16} Furthermore, the selected studies showed generally a high risk of bias (except 2 studies,^{8,13} which showed a medium risk of bias), limiting the strength of evidence.

An important variable that can constitute a bias is the operator's experience; only in 1 study, the extractions were performed by the same surgeon,⁸ while in most of the remaining ones, the extractions were performed by dif-

ferent surgeons with different levels of experience; these studies did not specify if the interventions were assigned to the surgeon randomly or if there was a correlation between the surgeon's experience and surgical difficulty.^{14–17} Brann et al. did not give information about the operator's experience.¹³

In 2 studies, the operator's experience was the major factor influencing the frequency of IAN and LN injury during lower third molar extraction.^{13,15} The uncontrolled application of force, low ability in the management of surgical instruments and the lack of experience could cause nerve injury.¹⁸

In 5 of the 6 studies, surgical difficulty was also analyzed with different classifications (Winter's classification, Wharfe's score and Pell and Gregory's classification).^{8,13–16} Only 3 studies comparing procedures under LA and GA in terms of surgical difficulty showed no substantial differences between the study groups.^{8,13,16} One of these studies did not show a relationship between nerve damage and the anesthetic modality¹⁶; the other 2 studies showed that GA seemed to increase the risk of developing IAN and LN lesions.^{8,13}

In the studies by Gülicher and Gerlach¹⁵ and by Nguyen et al.,¹⁷ patients were assigned to surgery under GA based on many factors, including the expected high level of surgical difficulty. In 2 studies, there could be observed a trend to direct patients to surgery under GA rather than LA.^{13,16} In fact, in the UK, dental procedures have been performed frequently under GA because of the preference of both surgeons and patients.⁵

A non-homogeneous distribution of surgical difficulty inside experimental groups could be the reason of bias in the evaluation of the influence of LA and GA on the development of nerve injury.⁸ Constantinides et al. supposed that the anesthetic regimen should not be chosen according to the surgeon's preference, but to the patient's choice or clinical characteristics (e.g., dental phobia).⁸ Such an attitude results in a better distribution of surgical variables, and consequently enables a more reliable comparison between groups, with a lower risk of bias.

The use of a lingual retractor is another parameter that can produce a bias. In particular, it regards the Howarth retractor, which is a narrow instrument used for lifting and retracting the surgical flap; it offers poor flap protection and is capable of exerting considerable force on LN, inducing injury, and the alteration or blockage of nerve conduction.^{16,17} The Howarth retractor was used in 3 studies.^{13,14,16}

Retraction has been shown to increase the number of temporary sensory LN injury cases due to neuropraxia. For this reason, many authors suggest avoiding the preparation and retraction of the lingual flap. No cases of permanent LN injury was found when the Howarth retractor was used.^{13,15,18,21}

Rehman et al. observed a total of 3 cases of IAN permanent injury, without distinguishing the anesthetic modality, while no permanent LN injury was observed.¹³ Hill et al. did not observe permanent nerve injury in their study.¹⁴ Both authors concluded that there was no significant difference between lower third molar surgery performed under LA or GA.^{13,14}

The number of cases of permanent injury after GA is greater in the studies by Gülicher and Gerlach¹⁵ and by Nguyen et al.¹⁷ Such data could be a consequence of the high number of extractions performed (1,106 and 11,599 teeth removed, respectively), which could increase the probability of adverse events.^{15,17}

Brann et al. elaborated some theories on a high risk of developing nerve injury after GA and hypothesized that procedures under GA could be complicated by the supine position or by the extent of mucoperiosteal stripping and bone removal.¹³ The same author suggests that the degree of surgical force may be greater under GA and that a conscious patient provides a series of signals to the surgeon, who tends to limit tissue retraction and surgical force, and thus decreases the risk of nerve injury.¹³

Based on the retrieved data, it is not possible to determine a correlation between the anesthetic modality and

nerve injury because of different variables that are related to each procedure of extraction and a high risk of bias across the studies.

The only certain indication described in the literature is that the prevalence of complications after dental procedures under GA have induced a reduction in the number of procedures performed with this anesthetic modality.^{13,22}

D'Eramo reported a mortality rate of 1:1,733,055 and a frequency of adverse events of 1:26,698 in patients undergoing GA for oral-maxillofacial surgery.²³ The most commonly observed adverse event is laryngospasm, present in 1 out of 833 patients (0.12%) treated under GA. The same author reported with a lower frequency the following: cardiac arrhythmias; bronchospasm; hypertension; hypotension; congestive heart failure; angina pectoris; myocardial infarction; nerve and/or cervical lesions associated with changes in the patient's position during anesthesia; phlebitis; insulin shock; and diabetic ketoacidosis.²³

Four of the 6 studies analyzed showed a greater incidence of neurological damage following the extractions performed under GA.

The quality and the number of studies on the topic discussed in the present review is low, and more investigations are necessary with better-quality studies.

Conclusions

In third molar surgery, LA should be preferred when it is possible because of the increased rate of complications under GA. However, GA remains an appropriate anesthetic modality in case of complex and long procedures, uncooperative patients, dental phobic patients, and patients with allergy to a local anesthetic.

Registration

The present systematic review was registered in the International Prospective Register of Systematic Reviews under No. PROSPERO CRD42021231823.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

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Assessment of stress burden among dental students: A systematic literature review and meta-analysis of data

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Abstract

Background. The education of dentists is associated with high levels of stress among students. This phenomenon is observed in many countries.

Objectives. The aim of the study was to sum up the knowledge about stress burden among dental students and to determine the causal factors on the basis of a quantitative systematic review of the literature.

Material and methods. The PubMed/MEDLINE and Cochrane Library databases were reviewed, and the literature was manually searched for the following keywords: ‘stress’; ‘dental student’; ‘dental education’; and ‘DES questionnaire’. The inclusion criteria for the systematic literature review were original papers and literature reviews, published after 1990, written in English, containing the analysis of the DES questionnaires, and on the topic of stress among students of dentistry. The inclusion criteria for the meta-analysis were publications with the same thematic structure of the DES questionnaire and with the number of survey participants provided.

Results. A total of 36 original papers were found, out of which 29 met the inclusion criteria, and thus were included into the systematic literature review analysis. Taking all the papers into consideration, the highest level of stress was caused by grades and examinations, and the atmosphere created by clinical professors. Nearly half of the studies demonstrated higher levels of stress in women. Also, students of higher years were characterized by higher stress indicators. The statistical analysis comprised data from 11 European universities that met the inclusion criteria. The highest mean scores were obtained for ‘performance pressure’, while the lowest mean scores for ‘relationship with sex/race’.

Conclusions. There was a great heterogeneity among the pooled estimates, which could be attributed to the varying levels of stress among dental students. The students’ ethnicity and cultural indicators have a decisive influence on the stress levels of dental students, not the characteristics of the field of study.

Keywords: stress, dental education, dental student, DES questionnaire

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Introduction

Stress, which in the early 1930s was defined by Hans Selye as a dynamic adaptive relation between the abilities of an individual and the requirements of a situation,^{acc.1} is now a significant part of our everyday lives. It partially provides stimulation that contributes to improved performance, but unfortunately, when this stimulation is prolonged or overlaps with another, it entails negative consequences, both emotional and somatic.² A recent report by the British Association for Counselling and Psychotherapy (BACP) states that in the student population, the number of people with emotional imbalance due to stress has increased significantly over the past few decades. The percentage of people who require the intervention of a therapist has been growing as well. One can dare to say that dental students, as compared to other fields of studies, are more vulnerable to prolonged stress, as they have to meet more than just theoretical requirements. Great emphasis is also put on clinical practical skills and a positive relationship with patients, which is indispensable in this profession.³ Additionally, personal issues may also affect a future doctor, which intensifies negative effects.

The research on the stress levels and the specific causes of stress among dental students was begun by Goldstein in 1979.⁴ He has been followed by other researchers. Garbee et al. developed a universal Dental Environment Stress (DES) questionnaire,⁵ which after being modified by Grandy, has become the most popular tool in monitoring the sources of stress among prospective dentists.^{6,7} Until now, it has been used in many studies around the world, often combined with other scales, such as the Perceived Stress Scale (PSS-10)⁸ and the Maslach Burnout Inventory (MBI).^{9,10} The DES questionnaire has been proven to cover all the most important aspects of the environment of dental students and can be successfully applied as an objective tool that points to the specific sources of psychological tension among students.

Originally, the DES questionnaire created by Garbee in 1980,⁵ and then modified by Grandy in 1989⁷ and by Westerman in 1993,⁶ included 38 questions referring in their thematic structure to the following areas: self-efficacy beliefs; performance pressure; faculty and administration; academic performance; patient-related aspects; study conditions; and relationship with sex/race.⁴⁻⁷ For each question beginning with the phrase "How often", a student answered with one numeric value. The scores were as follows: 1 – never; 2 – hardly ever; 3 – sometimes; 4 – quite often; and 5 – very often.

However, the researchers who used the abovementioned tool to measure stressors during dental studies have introduced their own amendments, which they considered appropriate due to cultural, social and economic differences between individual countries. Some questions have been added, others removed. In this way, a shortened DES-16 questionnaire was created, in which there were no questions about the aspects related to patient treatment.¹¹ It was

applied to students of lower years who had not had clinical classes yet.

The aim of the study was to sum up the knowledge about stress burden among dental students, as examined by the DES questionnaire, and to determine the causal factors on the basis of a quantitative systematic review of the literature and the meta-analysis of data.

Material and methods

Systematic literature review

The PubMed/MEDLINE and Cochrane Library databases were reviewed, and the literature was manually searched for the following keywords: 'stress'; 'dental student'; 'dental education'; and 'dental environment stress (DES) questionnaire'. The search was carried out independently by 2 researchers and was completed in December 2019. The inclusion criteria were original papers and literature reviews, published after 1990, written in English, containing the analysis of the DES questionnaires (with modifications), and on the topic of stress among students of dentistry. Study participants were undergraduate dental students, from the 1st to the 5th year of studies.

Meta-analysis of data

The PubMed/MEDLINE and Cochrane Library databases were reviewed, and the literature was manually searched according to the previously described principles. Due to the lack of the structural homogeneity of the DES questionnaire in particular versions, it was decided to perform a meta-analysis of the mean DES scores for each of the items included in the thematic structure of the DES questionnaire. After analyzing all available DES modifications, it was found that the greatest amount of research was based on the following thematic structure: self-efficacy beliefs; performance pressure; university rules and regulations; academic performance; patient-related aspects; study conditions; and relationship with sex/race.^{10,12} The inclusion criteria were publications with the same 7-point thematic structure of the DES questionnaire that contained the mean DES scores with standard deviation (*SD*) for each of the 7 items as well as the number of survey participants. Similarly to the systematic literature review, only original papers and literature reviews, published after 1990, written in English, containing the analysis of the DES questionnaires (with modifications), and on the topic of stress among students of dentistry were eligible. A small number of participants may present a risk of bias in a research paper. The meta-analysis of the scores was performed in the fixed-effects and random-effects models, depending on the result of the study heterogeneity test. Heterogeneity was determined with Cochran's *Q* test, which checks whether variability in the observed effect sizes or outcomes is larger than would be expected based

on sampling variability alone. A significant test suggests that the true effects or outcomes are heterogeneous. To assess the degree to which results from different studies were heterogeneous, the I^2 coefficient was used. The DerSimonian–Laird estimator was used for the random-effects model. Statistical tests were performed using the R software, v. 3.5.1, the Metafor package.¹³

Results

Systematic literature review

A total of 36 original papers were found, out of which 29 met the inclusion criteria, and thus were included into the analysis (Fig. 1). The results came from 17 European, 12 Asian, 11 North American, 1 South American, 1 African, and 1 Australian university.

Taking all the papers into consideration, the highest level of stress was caused (in descending order) by:

- grades and examinations;
- the atmosphere created by clinical professors; and
- the amount of the assigned classwork.

Nearly half of the studies demonstrated higher levels of stress in women. The year of studies was also found to be crucial – students of higher years were characterized by higher stress indicators as compared to students of lower years.^{1–12,14–33}

Meta-analysis of data

The statistical analysis comprised data from 11 European universities that met the inclusion criteria. The investigated students were from the universities of Manchester (UK), Belfast (UK), Helsinki (Finland), Amsterdam (the Netherlands), Cork (Ireland), Athens (Greece), Zagreb (Croatia), Ljubljana (Slovenia), Dublin (Ireland), Santiago de

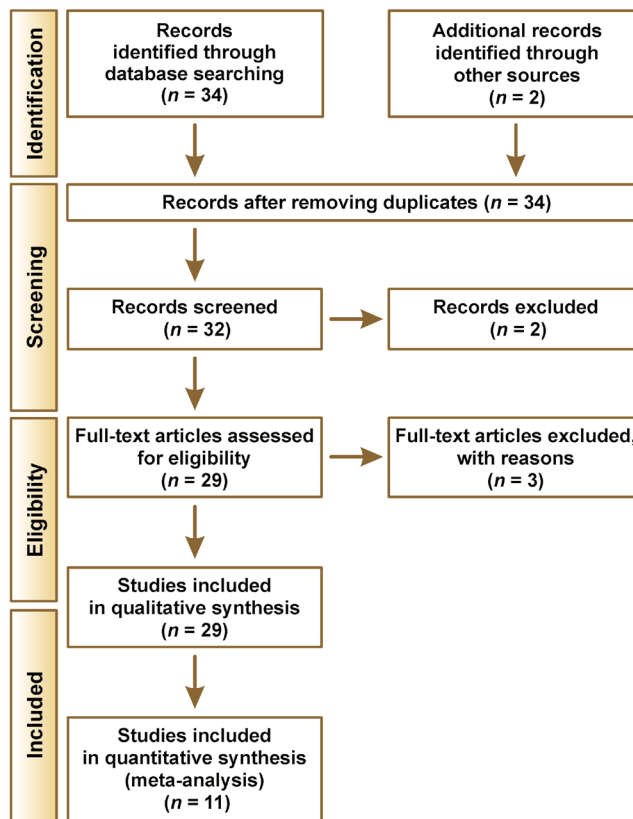


Fig. 1. PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flow diagram

Compostela (Spain), and Malmö (Sweden). The following items of the DES questionnaire were analyzed (Table 1)^{10,12}:

- self-efficacy beliefs;
- performance pressure;
- university rules and regulations;
- academic performance;
- patient-related aspects;
- study conditions;
- relationship with sex/race.

Table 1. Summary of the included studies

Subject of questions	Manchester UK	Belfast UK	Helsinki Finland	Amsterdam Netherlands	Cork Ireland	Athens Greece	Zagreb Croatia	Ljubljana Slovenia	Dublin Ireland	Santiago Spain	Malmö Sweden
Self-efficacy beliefs	2.04	2.40	1.67	1.67	2.00	2.73	2.59	2.66	2.61	2.58	2.32
Performance pressure	2.09	2.54	2.02	2.03	2.27	2.37	2.74	2.74	2.8	2.98	2.38
University rules and regulations	1.89	1.25	1.71	2.69	1.81	2.31	2.39	2.36	2.39	2.20	1.99
Academic performance	1.87	2.28	1.64	1.82	1.95	2.27	2.18	2.21	2.28	2.59	2.17
Patient-related aspects	2.34	1.96	2.00	2.47	2.31	2.55	2.20	2.23	2.25	2.06	1.99
Study conditions	2.77	2.55	1.95	2.15	2.73	2.74	2.71	2.61	2.52	2.50	2.21
Relationship with sex/race	1.43	1.11	1.15	1.19	1.33	–	–	–	–	–	–
Number of participants	32	22	17	39	22	580	372	131	153	110	146

Numbers describe the arithmetic means of the values the students assigned to each question from the given thematic structure.

The heterogeneity tests demonstrated a significant heterogeneity of the studies ($p < 0.001$; $p = 0.034$ was only for the item 'relationship with sex/race'); therefore the scores were obtained from the random model. The highest mean scores were obtained for 'performance pressure' (2.47) and 'study conditions' (2.50), while the lowest mean scores for 'relationship with sex/race' (1.21). The highest heterogeneity index I^2 was recorded for the item 'performance pressure' ($I^2 = 95.8\%$) and the lowest for 'relationship with sex/race' ($I^2 = 61.6\%$). The results are graphically presented in Fig. 2–8.

For the 'self-efficacy beliefs' item, the mean result was 2.32 with 95% confidence interval (CI) 2.15–2.50, which means that only 5% of the results were beyond the stated norm. However, the coefficient of heterogeneity I^2 was 94.9%, which suggests a large diversity of the trait and proves the heterogeneity of the studied population.

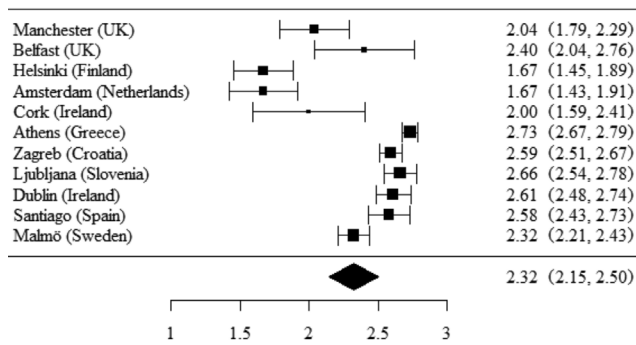


Fig. 2. Self-efficacy beliefs ($M = 2.32$ (95% CI: 2.15–2.50); $I^2 = 94.9\%$)
M – mean; *CI* – confidence interval; I^2 – coefficient of heterogeneity.

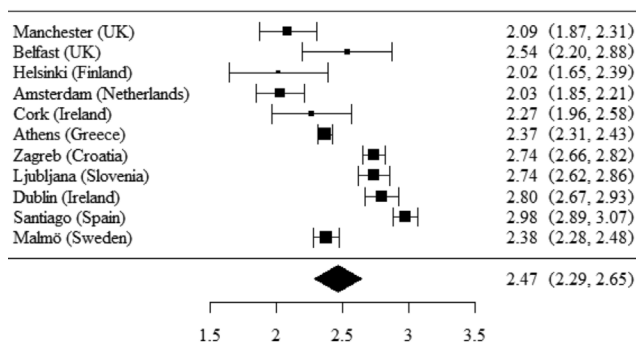


Fig. 3. Performance pressure ($M = 2.47$ (95% CI: 2.29–2.65); $I^2 = 95.8\%$)

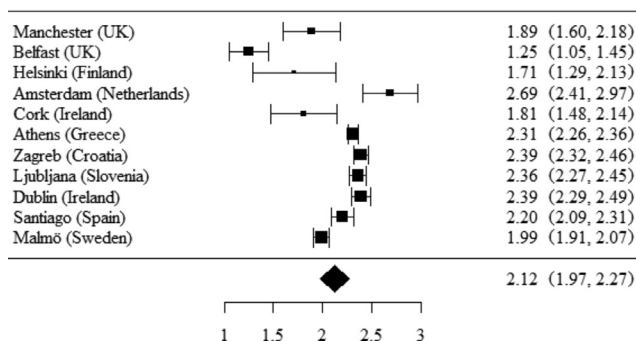


Fig. 4. University rules and regulations ($M = 2.12$ (95% CI: 1.97–2.27); $I^2 = 95.0\%$)

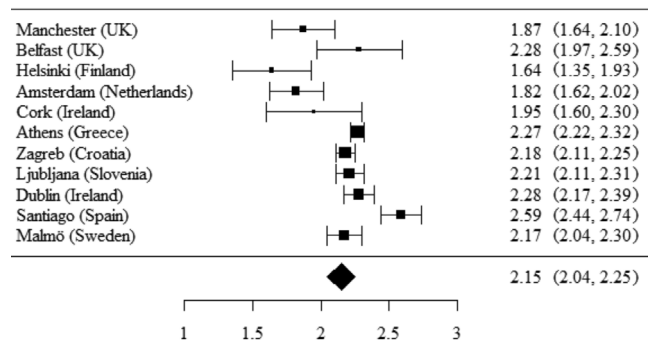


Fig. 5. Academic performance ($M = 2.15$ (95% CI: 2.04–2.25); $I^2 = 86.4\%$)

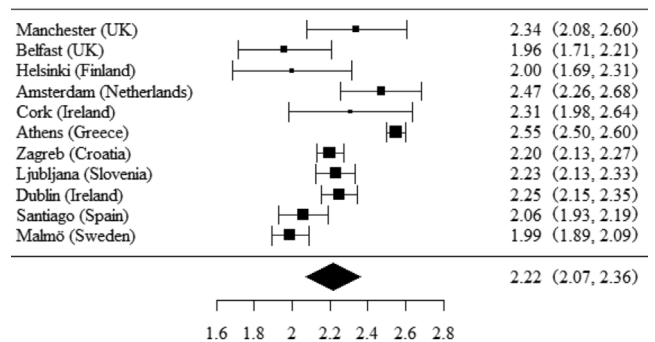


Fig. 6. Patient-related aspects ($M = 2.22$ (95% CI: 2.07–2.36); $I^2 = 94.3\%$)

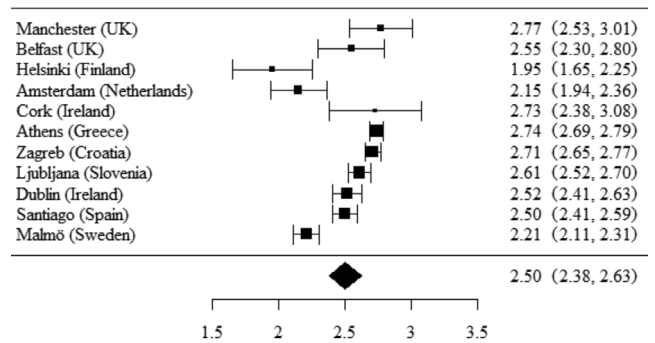


Fig. 7. Study conditions ($M = 2.50$ (95% CI: 2.38–2.63); $I^2 = 93.3\%$)

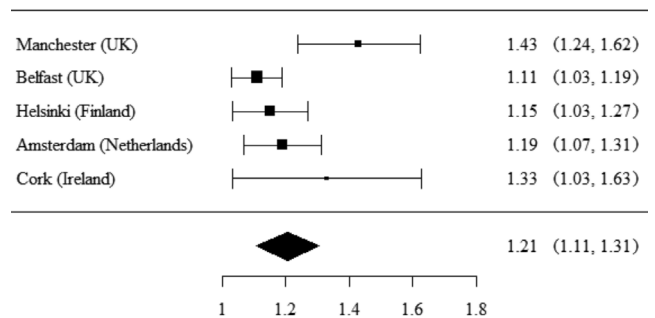


Fig. 8. Relationship with sex/race ($M = 1.21$ (95% CI: 1.11–1.31); $I^2 = 61.6\%$)

For the 'performance pressure', 'university rules and regulations', 'academic performance', 'patient-related aspects', and 'study conditions' items, the results were similar. 95% CIs showed little statistical dispersion of the data, although the coefficients of heterogeneity were high (from 86.4% to 95.8%).

Only in the item 'relationship with sex/race', less heterogeneity of the results was noted. However, it was related to the smaller number of students surveyed, as only in 5 universities, the DES questionnaire contained questions related to this point.

Discussion

Despite searching 2 databases and, additionally, a manual literature search, there is a risk of not including in the review and meta-analysis all available studies using the DES questionnaire. Access to the content of journals that are not included in the PubMed and Cochrane Library databases may be limited.

Furthermore, despite the seeming homogeneity of the studied group of students, the cultural, ethnic and, what is equally important, economic differences are enormous. Therefore, comparing reports from different countries should be done with great caution.

For instance, scores from the University of Lagos in Nigeria completely differ from others.¹⁴ The authors of the study, being familiar with the economic situation of the country and the reality of studying, extended the DES questionnaire with questions regarding the availability of clinical materials and textbooks, the living conditions of future dentists as well as the problem of facing possible unemployment despite obtaining higher education. The authors' predictions proved to be accurate. One of the biggest stressors turned out to be difficulties in obtaining clinical materials and textbooks in order to meet clinical requirements and to provide oneself with a favorable learning environment. The level of stress resulting from these difficulties was 3.82 for the male group and 3.77 for the female group. In comparison with the highest value obtained in the present meta-analysis (2.50 for 'study conditions'), it was at a very high level. Grades and examinations were often beyond the top 5 factors causing the greatest stress.¹⁴

For students in countries where university education is compulsorily payable, the item 'financial responsibility' was scored higher among the stress indicators as compared to students from universities with no tuition fee.^{2,12} For example, Greek students whose university is subsidized had an average DES score of 2.05.¹² On the other hand, students in San Diego, USA, who are charged the tuition fee, had a mean DES score of 2.70, whereas at the University of Trinidad and Tobago, West Indies, the highest mean DES score for 'financial responsibility' was found among 4th year students and amounted to 3.33. It seems quite obvious, since the tuition fee significantly increases the financial expenditure, which already includes accommodation, meals, textbooks, etc. For many students this means that they have to take a loan and pay off the debt after gradu-

ating. However, it is rightly suspected that this factor undergoes great individual variability and is influenced by the financial situation of particular students. For some students, the tuition fee and paying for convenient accommodation will not be the slightest problem, while for others, even the purchase of textbooks may be a considerable economic stressor. In such cases, university aid programs and scholarships for students may prove to be a factor that reduces the stress related to financial responsibility.

Being a woman is not a universal stress indicator, either. It mainly occurs at universities where male students significantly outnumber female students.^{1,14,29} Most of the differences in the level of stress between the sexes are not significant. At the King Saud University, Riyadh, Saudi Arabia, women scored higher in each of the thematic structure items of the DES questionnaire, but a statistically significant difference was observed only for the 'workload' item (3.28 for males and 3.56 for females; $p < 0.0001$). Religion and the traditions of individual countries also have a significant impact on this matter. Gorter et al. predict that these differences will diminish as women play more and more assertive roles in the society.⁹

The type of curriculum also has a considerable influence on the level of stress and its various factors. Polychronopoulou et al. showed that problem-based learning (PBL) compared to the traditional lecture-based learning (LBL) curriculum resulted in much lower DES values for the questions related to self-efficacy.¹² At Malmö University, Sweden, with the PBL curriculum type, the mean DES score for 'self-efficacy beliefs' was 2.32, whereas at the University of Athens, Greece, with the LBL curriculum type, the mean DES score was 2.73 for the same item of thematic structure.¹² This important finding is in line with the assumptions of the PBL method. Increased capabilities of independent assessment, better interaction of peers and collaboration between prospective doctors are integral parts of most PBL modules.¹⁶ Moreover, a reverse association between the teaching methods and workload seems significant. For students following the PBL program, the item 'excess workload' was found lower among the stress indicators than for students implementing the traditional curriculum. The way of delivering content in the PBL module is more intuitive and it is easier to learn in a shorter time. In PBL, information is conceptually organized and interwoven into clinical scenarios, thus facilitating the education process in a constructive way, with emphasis on self-learning by students and greater freedom as to how and when students access medical information. Similarly, positive effects were observed in the fields of patient treatment and clinical training, which increases the value of problem-based education in preparing a student to meet patients and encounter real therapeutic problems.¹⁷

The literature review comprised papers that applied the DES questionnaire as an objective tool pointing to the specific sources of psychological tension among students. However, the questionnaire has many limitations. Firstly, it has been constantly altered and updated to match its content to the local reality. It has resulted in the changed number of questions and thematic structure in each paper evaluated, which made it difficult to perform a meta-analysis of the available data. The original language of the DES questionnaire is English. The translations in different countries slightly vary from the source text, which also hinders an objective comparison of the test results. As noted by Sanders and Lushington, the psychometric properties of the DES questionnaire have not been systematically verified with reference to the standard stress measure, nor was the reliability of the scale tested in the test–retest evaluation.¹⁸ There are no specific standards or survey instructions, either. Strictly speaking, the DES questionnaire in fact is not a measure of stress among dental students, but rather a tool for identifying contextual stressors.¹⁹

The heterogeneous structure of the DES questionnaire hindered the statistical meta-analysis of all available data. In each paper, the DES questionnaire was slightly modified. It was only possible to analyze scores from 11 European universities, in which the identical thematic structure of the DES was found. The results of the statistical analysis indicate ‘performance pressure’ and ‘study conditions’ as the 2 biggest stressors. This coincides with general conclusions from the literature review and appears to be natural for students, whose responsibility at this stage of life is connected with gaining as much knowledge as possible and passing the examinations which authorize them to pursue the chosen profession. A positive surprise was a low score of the stressor of ‘relationship with sex/race’. In addition, this item demonstrated the lowest heterogeneity rate, which means that the answers were relatively unanimous and the scores from various universities did not differ significantly from each other. This shows the high personal culture of students and the resulting lack of prejudice in the context of sex or race. The indirect results of stressors such as ‘patient-related aspects’ or ‘self-efficacy beliefs’ suggest that students who undertake dentistry studies are aware of the need for frequent contact with patients and the correct implementation of the treatment procedures related to treatment planning and manual skills. The stress caused by the above situations is not significant, but it occurs, which can have a stimulative effect and lead to improved performance. However, the high heterogeneity of scores, being in the highest range of $I^2 = 75\text{--}100\%$ (except for the item ‘relationship with sex/race’), seems to be staggering. This phenomenon is present even despite the relative homogeneity of the study group associated with a similar age and studying in European cities. According to the information provided by the textbook by Cochrane, in such cases, the combined data

analysis can only be carried out with special caution and the objectivity of interpretation.³⁴

Students around the world are subject to a relatively high level of stress. The stressors perceived by students vary considerably depending on the institution and are associated with individual institutional parameters. Prolonged emotional exhaustion can quickly lead to professional burnout. The didactic role of the university seems indispensable in such cases. It is essential to identify the potential sources of stress to be able to effectively eliminate or minimize them. In some units, structural changes may be necessary (updating the curriculum, implementing student-centered methods, lowering educational costs), whereas in others, minor modifications will be sufficient, such as providing individual advice by academic teachers or senior students.¹⁴ Unfortunately, it is not possible to eliminate all stressors at the university. Young people need to learn how to deal with problems, too. Nevertheless, there are some students who require help in this regard. Therefore, the next primary aim of research using the DES questionnaire should be to search for students who, without external support, could not emotionally cope with the excess of stress. University support in this area shall be seen as a good investment for the benefit of students, future dentists and their patients.⁹

Conclusions

The statistical meta-analysis showed that the greatest stressors for the entire study group of dentistry students were the study conditions and performance pressure. However, the individual examination of each study highlighted the decisive influence of ethnic origin and cultural indicators on the greatest stressors in individual countries.

Ethics approval and consent to participate

I state that the study protocol and the informed consent procedure were approved by the institutional Ethics Committee.



Data availability

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

Consent for publication

Not applicable.

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Minimally invasive surgical techniques for the treatment of different types of isolated intrabony defects: A narrative review

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Abstract

The aim of this literature review was to present minimally invasive surgical techniques (MISTs) for the regeneration of intrabony defects and the impact of these surgical procedures on clinical outcomes. Less invasive surgical procedures lead to a more uneventful postoperative healing and reduce patient morbidity. The introduction of these techniques together with the use of magnification tools enables gentle tissue manipulation with performing minimal incisions and flap elevations in the field of surgical treatment of intrabony defects. Minimally invasive surgical techniques induce minor surgical trauma and improve the wound stability with favorable results in terms of clinical outcomes and the patient's comfort. The defect anatomy, patient-centered factors and the various biomaterials applied are considered. Recent evidence concludes that the adjunctive use of regenerative materials seems to have a less determinant effect on the clinical performance of minimally invasive surgical techniques. In addition, more studies are required to investigate the clinical efficacy of these surgical techniques in the treatment of intrabony defects in comparison with the conventional papilla preservation flap techniques.

Keywords: review, surgical treatment, periodontal, attachment loss, intraosseous defects

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Introduction

Over the last decades, less invasive surgical procedures that could lead to a more uneventful postoperative healing and reduce patient morbidity has been one of the most important objectives of the clinicians. The introduction of the operating microscope in surgery served this purpose and gave the surgeons the chance to follow the principles of the conventional surgical procedures in a less extensive and more precise way.^{1,2} The potential of gentle tissue manipulation under magnification was called minimally invasive surgery (MIS)³ and gradually, the concept of minimal surgical trauma was applied in the field of periodontal surgery.⁴

A periodontal lesion extends apically toward the tooth root, causing the loss of the periodontal support and the destruction of the alveolar bone. As a result, osseous defects occur. Their morphology depends mainly on the presence of the microbial plaque and local anatomical factors. Infrabony lesions represent vertical defects, in which the base of the pocket is detected apical to the level of the alveolar crest.⁵ Periodontitis is an infectious disease that may also be correlated with systemic illnesses, such as cardiovascular diseases⁶ and preeclampsia.⁷ The conservative non-surgical periodontal therapy with scaling and root planing alone or in combination with topical and/or systemic adjuncts, like subantimicrobial-dose doxycycline, antimicrobials, chlorhexidine chips, and melatonin, leads to favorable clinical outcomes in terms of the clinical attachment level (CAL) gain.^{8,9} However, infrabony lesions may be related to a higher risk of progression of periodontal disease, as they tend to present deeper residual pocket depth (PD) after non-surgical periodontal therapy.⁵ Thus, a variety of surgical approaches and regenerative materials have been developed in order to treat the residual defects.¹⁰ The conventional flap surgery in combination with regenerative materials, such as membrane barriers, bone grafts and enamel matrix derivatives (EMDs), has proven to be very effective in the regeneration of intrabony defects.^{11–13}

Recently, progress in the field of magnification has led to the development of new less invasive periodontal surgery techniques. Minimally invasive surgical techniques (MISTs) are characterized by small incisions, limited flap reflection and suturing for primary wound healing. Based on the principle of the papilla preservation techniques,¹⁴ which is to maintain the interdental papilla tissue as intact as possible, researchers have introduced and improved new surgical approaches for periodontal regeneration.^{15–19} Less invasive surgical procedures demonstrated favorable clinical outcomes in the treatment of intrabony defects, even in esthetic areas.^{16,20} In addition, the minimal surgical trauma and the reduced chair time made them better accepted by patients, and improved patient perception over surgical procedures.²⁰ These new perspectives has led more clinicians to apply MISTs in the treatment of intrabony lesions, when it was indicated.

The aim of this literature review was to present MISTs for the regeneration of intrabony defects and the impact of these surgical procedures on clinical outcomes.

Literature search

A literature search was performed in MEDLINE via the PubMed database for articles published until August 2021. The database was searched using search terms and free text terms, in different combinations.

To be included in the review, studies had to be written in the English language, be published in an international peer-reviewed journal, and present (MISTs) and periodontal regeneration for the treatment of intrabony defects.

The assessment of the titles, abstracts and full texts of the papers was performed by the 2 authors. Finally, in the present review, 6 randomized clinical trials (RCTs), 10 case series studies and 1 prospective cohort study were included. All studies applied MISTs and presented a minimum of 6 months of follow-up. Clinical attachment level, PD and recession (REC) were reported as clinical outcomes (Table 1).

Minimally invasive surgical techniques

Minimally invasive surgical (MIS) approach

The minimally invasive surgical (MIS) approach was first introduced by Harrel.^{15,16} The initial incision is made intrasulcularly around the teeth adjacent to the osseous defect, with the blade parallel to the long axis of the root. Subsequently, an incision is performed, usually on the lingual aspect, to connect the 2 intrasulcular incisions. The interdental papilla is sharply dissected from the underlying bone and small flaps are reflected buccally and lingually. Flap reflection is followed by the removal of the connective and granulation tissue within the osseous defect. This is performed with the use of a blade and small curettes as well as an ultrasonic scaler, which is also used for the initial root debridement. The remaining granulation tissue is shredded and removed with a mechanical instrument, and root planing is refined with finishing burs. In order to obtain the primary closure of the wound, a vertical mattress suture is used to close the flaps.^{15,16,20}

Minimally invasive surgical technique (MIST)

In 2007, Cortellini and Tonetti proposed the minimally invasive surgical technique (MIST)¹⁸ based on the MIS approach combined with the modified papilla preservation flap technique (MPPT)²¹ or the simplified papilla preservation flap technique (SPPT).²² The width of the interdental space determines the choice of the surgical approach; if it is ≤ 2 mm, SPPT is used, whereas if it is > 2 mm, MPPT is per-

Table 1. Characteristics of the selected studies

Authors, year	Study design	Treatment	Follow-up	Parameters	Results
Harrel et al. 2005 ²⁹	case series	MIS + EMD	11 months	PD CAL REC	Δ PD: -3.56 ± 1.31 CAL gain: 3.57 ± 1.75 Δ REC: -0.01
Cortellini and Tonetti 2007 ¹⁸	case series	MIST + EMD	12 months	PD CAL REC defect resolution	Δ PD: -4.8 ± 1.8 CAL gain: 4.8 ± 1.9 Δ REC: -0.1 ± 0.9 bone fill: 88.7 ± 20.7
Cortellini and Tonetti 2007 ²³	case series	MIST + EMD	12 months	PD CAL REC defect resolution VAS	Δ PD: -5.2 ± 1.7 CAL gain: 4.9 ± 1.7 Δ REC: 0.4 ± 0.7 bone fill: 77.6 ± 21.9 VAS: pain 19 ± 10 discomfort 28 ± 11
Cortellini et al. 2008 ³⁴	case series	MIST + EMD	12 months	PD CAL REC defect resolution VAS	Δ PD: -4.6 ± 1.3 CAL gain: 4.4 ± 1.4 Δ REC: 0.2 ± 0.6 bone fill: 83 ± 20 VAS: pain 19 ± 9 perception of the hardship of the surgical procedure 24 ± 12 discomfort 21 ± 10
Cortellini and Tonetti 2009 ¹⁹	case series	M-MIST	12 months	PD CAL REC	Δ PD: -4.6 ± 1.5 CAL gain: 4.5 ± 1.5 Δ REC: 0.1 ± 0.3
Trombelli et al. 2009 ²⁴	case series	SFA + HA + collagen	10 \pm 3 months	PD CAL REC	Δ PD: -5.2 ± 2.6 CAL gain: 4.8 ± 2.7 Δ REC: 0.4 ± 1.5
Harrel et al. 2010 ³³	case series	MIS	6 years	PD CAL REC	Δ PD: -3.78 ± 2.12 CAL gain: 3.70 ± 1.15 Δ REC: 0.004 ± 0.56
Trombelli et al. 2010 ⁴¹	RCT	test: SFA + HA/GTR control: SFA	6 months	PD CAL REC	test: Δ PD: -5.3 ± 2.4 CAL gain: 4.7 ± 2.5 Δ REC: 0.4 ± 1.4 control: Δ PD: -5.3 ± 1.5 CAL gain: 4.4 ± 1.5 Δ REC: 0.8 ± 0.8
Cortellini et al. 2011 ³⁶	RCT	group 1: M-MIST group 2: M-MIST + EMD group 3: M-MIST + EMD + BMDX	12 months	PD CAL REC defect resolution	group 1: Δ PD: -4.4 ± 1.6 CAL gain: 4.1 ± 1.4 Δ REC: 0.3 ± 0.6 bone fill: 77 ± 17 group 2: Δ PD: -4.4 ± 1.2 CAL gain: 4.1 ± 1.2 Δ REC: 0.3 ± 0.5 bone fill: 71 ± 18 group 3: Δ PD: -4.0 ± 1.3 CAL gain: 3.7 ± 1.3 Δ REC: 0.3 ± 0.7 bone fill: 78 ± 27
Ribeiro et al. 2011 ³⁷	RCT	test: MIST + EMD control: MIST	6 months	PD CAL REC	test: Δ PD: -3.56 ± 2.07 CAL gain: 3.02 ± 1.94 Δ REC: 0.46 ± 0.87 control: Δ PD: -3.50 ± 0.88 CAL gain: 2.82 ± 1.19 Δ REC: 0.54 ± 0.58

Authors, year	Study design	Treatment	Follow-up	Parameters	Results
Mishra et al. 2013 ³⁰	RCT	test: M-MIST + rhPDGF-BB control: M-MIST	6 months	PD CAL REC defect resolution	test: Δ PD: -4.18 ± 0.60 CAL gain: 3.00 ± 0.89 Δ REC: 0.82 ± 0.60 bone fill: 36 ± 18 control: Δ PD: -3.82 ± 0.87 CAL gain: 2.64 ± 0.67 Δ REC: 0.55 ± 0.52 bone fill: 35 ± 11
Harrel et al. 2014 ¹⁷	case series	V-MIS	6 months	PD CAL REC	Δ PD: -3.88 ± 1.02 CAL gain: 4.04 ± 1.38 Δ REC: -0.13 ± 0.61
Harrel et al. 2016 ⁴⁰	case series	V-MIS	12 months	PD CAL REC	Δ PD: -4.11 ± 0.98 CAL gain: 4.58 ± 1.19 Δ REC: -0.48 ± 0.65
Harrel et al. 2017 ³⁹	case series	V-MIS	36 months	PD CAL REC	Δ PD: -3.80 ± 1.18 CAL gain: 4.16 ± 1.18 Δ REC: -0.36 ± 0.64
Ahmad et al. 2019 ³¹	RCT	test: M-MIST + PRF control: M-MIST	6 months	PD CAL REC defect resolution	test: Δ PD: -4.12 ± 0.95 CAL gain: 4.00 ± 1.06 Δ REC: 0.06 ± 0.25 bone fill: 37.4 ± 10.0 control: Δ PD: -4.18 ± 0.98 CAL gain: 4.00 ± 1.09 Δ REC: 0.06 ± 0.25 bone fill: 33.8 ± 11.1
Mizutani et al. 2021 ³⁵	prospective cohort study	DM group: MIST/M-MIST + EMD non-DM group: MIST/M-MIST + EMD	3 years	PD CAL REC defect resolution	DM group: Δ PD: -4.5 ± 1.4 CAL gain: 3.8 ± 1.1 Δ REC: 1.1 ± 0.9 bone fill: 58.3 ± 10.4 non-DM group: Δ PD: -4.7 ± 1.4 CAL gain: 4.1 ± 1.1 Δ REC: 0.6 ± 1.0 bone fill: 65.5 ± 18.8
Liu et al. 2022 ³⁸	RCT	test: MIST + DBBM + collagen control: MIST	12 months	PD CAL REC defect resolution	test: Δ PD: -2.31 ± 1.47 CAL gain: 2.00 ± 1.38 Δ REC: 0.31 ± 0.93 bone gain: 3.85 ± 1.69 control: Δ PD: 2.50 ± 1.22 CAL gain: 2.53 ± 1.80 Δ REC: -0.03 ± 1.19 bone gain: 3.00 ± 1.56

RCT – randomized clinical trial; MIS – minimally invasive surgery; EMD – enamel matrix derivative; MIST – minimally invasive surgical technique; M-MIST – modified minimally invasive surgical technique; SFA – single-flap approach; HA – hydroxyapatite; GTR – guided tissue regeneration; BMDX – bone mineral-derived xenograft; rhPDGF-BB – recombinant human platelet-derived growth factor BB; V-MIS – videoscope-assisted minimally invasive surgery; PRF – platelet-rich fibrin; DM – diabetes mellitus; DBBM – deproteinized bovine bone mineral; PD – pocket depth; CAL – clinical attachment level; REC – recession; VAS – visual analog scale. The PD, CAL and REC values are provided in millimeters [mm], bone fill as percentage [%], and bone gain also in millimeters [mm].

formed. The simplified papilla preservation flap technique consists in an oblique incision close to the buccal side of the interdental papilla col, while in MPPT, a horizontal incision at the buccal side of the papilla is performed. Two intrasulcular incisions extended buccally and lingually to the 2 teeth neighboring the defect are made. The mesiodistal extension of the intrasulcular incisions is as limited as possible, and 2 small full-thickness flaps are reflected to expose 1–2

mm of the bone crest. Vertical releasing incisions are made when better reflection of the flaps is demanded. The defect debridement and root planing follow with the use of small curettes and power-driven instruments. Finally, the suturing of the flaps is achieved with a single modified internal mattress suture, while the vertical incisions, if performed, are sutured with simple passing sutures. The surgical procedures are performed with the use of an operative microscope.^{18,20,23}

Modified minimally invasive surgical technique (M-MIST)

In order to further reduce surgical trauma, Cortellini and Tonetti described the modified minimally invasive surgical technique (M-MIST) in 2009.¹⁹ This technique is an improved and less invasive version of MIST. As in MIST, MPPT or SPPT is used to access the defect adjacent to the interdental papilla. The interdental incision is extended intrasulcularly at the buccal aspect of the involved teeth. A very small triangular flap is reflected to expose the coronal part of the bone crest. The granulation tissue filling the defect is moved away with the use of a micro-blade to split it from the interdental tissues. The incisions are not extended to the palatal tissues. After the removal of the granulation tissue underneath the papilla, the defect debridement and root planing are performed with the use of micro-curettes and power-driven instruments. Access is achieved through a buccal window to thoroughly debride the defect and the roots, with respect to the papillary fibrous attachment. Afterward, a single modified internal mattress is performed to close the flap. All the surgical procedures are carried out with the aid of an operative microscope.^{19,20}

Single-flap approach (SFA)

In the same year when M-MIST was introduced by Cortellini and Tonetti (2009), a similar surgical technique was proposed by Trombelli et al.²⁴ This technique consists in the limited elevation of a mucoperiosteal flap buccally or lingually, depending on the extension of the defect. Using a periodontal probe, the extension and the morphology of the defect are evaluated. Subsequently, sulcular incisions are performed, following the gingival margin of the teeth neighboring the surgical area. Following the contour of the underlying bone crest, a diagonal or horizontal butt-joint incision is made. The spot of this incision depends on the height of the interdental tissues. Taking this into account, the higher the interdental papilla is, the more apically the incision is performed, but always at least 1 mm coronal to the bone crest. In this way, an envelope flap, without the releasing incisions, is elevated and the supracrestal soft tissues as well as the rest of the papilla remain undetached. After the reflection of the flap, the debridement of the defect and the instrumentation of the roots follow, using curettes and mechanical instruments. For the reposition of the flap, firstly, a horizontal internal mattress suture is placed, and then a second vertical or horizontal internal mattress suture, in a more coronal spot of the flap and the papilla, is performed to achieve primary closure. The surgical procedures are carried out with the aid of $\times 2.5$ magnifying loupes.²⁴

Videoscope-assisted minimally invasive surgery (V-MIS)

The principal features of MISTs are small incisions and tiny flaps. Although these techniques limit surgical trauma, clinicians need to deal with a smaller surgical area than the one created in the conventional flap access. For this reason, Harrel in 1995 performed MIS with the aid of surgical telescopes (loupes),¹⁵ and later Cortellini and Tonetti introduced MIST with the use of the surgical microscope.¹⁸ Nevertheless, the inadequate magnification of the loops and the impractical use of the surgical microscope in clinical practice made MISTs less acceptable to the clinicians. To overcome this issue, Harrel in 2013 suggested the use of a videoscope flexible insertion tube with a digital camera to better visualize the surgical site.²⁵ A flow of gas over the end of the videoscope keeps away blood and debris, and prevents the lens from being blurred through the surgical procedure, providing a clear image of the surgical area on the monitor. In addition, a carbon fiber retractor makes the management of the soft tissues easier. Access to the defect is achieved with MIS, but only a tiny buccal or lingual split-thickness flap is performed, without the use of blunt dissection. The videoscope is placed at the defect and the removal of the granulation tissue as well as the root debridement follow. These procedures are meticulously performed, as the videoscope allows better visualization of the area. Finally, a single modified vertical mattress suture is positioned at the base of the papilla, while the tip of the papilla is approximated with the use of a wet gauze and finger pressure.^{17,25}

Minimally invasive surgical techniques (MISTs) and periodontal regeneration of intrabony defects

The successful regeneration of intrabony defects depends mainly on 2 factors – the surgical technique and the selected biomaterials.¹³ Since MISTs were introduced in the periodontal regenerative surgery, they have been combined with a variety of biomaterials, such as EMDs, bone grafts (demineralized freeze-dried bone allografts (DFDBA), xenografts) alone or with barrier membranes, as well as with growth factors and platelet-rich fibrin (PRF).^{18,24,26–31} Several clinical studies have been conducted aiming to evaluate the efficacy of MISTs with or without the adjunctive use of biomaterials, and define the best treatment modality for intrabony defects (Table 1).

The enamel matrix derivative Emdogain[®] has been widely used for the regeneration of intrabony defects alone or in combination with other biomaterials and different surgical techniques, leading to various clinical outcomes.³² Harrel et al. in 2 studies with an 11-month and

a 6-year follow-up, evaluated the use of enamel matrix proteins in conjunction with the MIS approach.^{29,33} A total of 160 sites of intrabony defects in 16 patients were treated and the results showed significant improvement in PD and CAL as compared to baseline. Specifically, at 11 months, changes in PD, CAL and REC were 3.56 mm, 3.57 mm and 0.01 mm, respectively, while at 6 years, the differences were not statistically significant. The authors concluded that clinical outcomes remained stable over the 6-year period and underlined favorable effects for the patient's esthetics, as they observed minimal gingival recession.^{29,33}

In a series of studies, Cortellini and Tonetti evaluated the clinical efficacy and the patient perception of MISTs in conjunction with the application of EMD in the treatment of isolated and multiple adjacent deep intrabony defects.^{18,23,34} Clinical outcomes were assessed at a 1-year follow-up, the single defects resulted in CAL gain of 4.9 ± 1.7 mm, associated with 3.0 ± 0.6 mm of residual PD and an increase in REC of 0.4 ± 0.7 mm,^{18,23} whilst the multiple defects resulted in CAL gain of 4.4 ± 1.4 mm, associated with 2.5 ± 0.6 mm of residual PD and an increase in REC of 0.2 ± 0.6 mm.³⁴ The authors concluded that MISTs combined with EMD could be applied successfully for these defects, and that it was an advantageous procedure in terms of patient morbidity.^{18,23,34} In a recent prospective cohort study, the clinical results of MISTs combined with EMD were also evaluated for the treatment of isolated intrabony defects in 10 patients with diabetes mellitus (DM) type 2, who completed a 3-year follow-up.³⁵ The mean PD reduction, CAL gain and REC increase were similar to those of the non-DM group and statistically different as compared to baseline after 3-year observation. Improvement in the radiographic defect depth and the radiographic bone fill were also comparable between the 2 groups. Thus, the application of MISTs combined with EMD could lead to favorable clinical results regardless of the presence of DM, although a larger number of cases would be preferable to validate this conclusion.³⁵ On the other hand, in an RCT where MISTs alone were compared to MISTs + EMD and MISTs + EMD + bone mineral-derived xenograph (BMDX) for the treatment of isolated interdental intrabony defects, it was shown that the additional application of regenerative materials did not improve the results, although, as it was emphasized, the power of the study was limited to detect a difference of 0.96 mm.³⁶

In a randomized, prospective, controlled clinical study, Ribeiro et al. compared MISTs + EMD and MISTs alone for the treatment of intrabony defects in a group of 14 patients.³⁷ Similar and significant results in terms of changes in PD, CAL, REC, and the radiographic parameters were demonstrated in the 2 groups at 3 and 6 months of follow-up. Thus, it was shown that in this type of bony defects, EMD did not offer an additional benefit to MISTs.³⁷ In

another more recent RCT, Liu et al. compared the efficacy of MISTs alone and MISTs + deproteinized bovine bone mineral (DBBM) + collagen membrane in the treatment of isolated 1-, 2- and 3-walled defects in 2 groups of 15 and 16 patients, respectively, who attended a 1-year follow-up.³⁸ Both groups noted significant CAL gain and bone fill after 1-year evaluation. Nevertheless, the authors failed to find any significant differences in terms of PD reduction and CAL gain as well as in radiographic bone gain between the 2 groups. These results indicate that the use of DBBM + collagen membrane does not have any additional effect on 1-year outcomes.

Videoscope-assisted minimally invasive surgery (V-MIS) in combination with EMD and DFDBA was also performed for the regenerative treatment of 1-, 2- and 3-walled defects.^{17,39,40} These studies demonstrated statistically significant improvement of the clinical measures as compared to baseline at 6 months, 12 months and 36 months postoperatively.^{17,39,40} It is worth reporting that V-MIS led to minimal REC and, in some cases, the soft tissue height increased as compared to baseline. These results could be explained by the fact that with the use of the videoscope, tiny incisions and more effective root debridement can be insured.¹⁷

Moreover, MISTs were applied in conjunction with guided tissue regeneration (GTR) and a hydroxyapatite (HA)-based biomaterial.^{24,41} Twenty-four intraosseous defects were treated with MISTs + HA/GTR or MISTs alone, and clinical outcomes in terms of PD reduction, CAL gain and REC increase were assessed at 6 months. It was concluded that MIST with and without HA/GTR should be considered as a valuable minimally invasive approach in the treatment of deep intraosseous periodontal defects.⁴¹

Minimally invasive surgical techniques have also been evaluated with growth factors. In a randomized, controlled, two-armed study, Mishra et al. assessed the impact of the addition of the recombinant human platelet-derived growth factor BB (rhPDGF-BB) gel on the efficacy of MISTs.³⁰ The results of the study showed significant PD reduction and CAL gain as compared to baseline in both groups – MISTs alone and MISTs + rhPDGF-BB – but no statistically significant intergroup differences were observed at 6 months postoperatively. Thus, improvement in the clinical and radiographic parameters was attributed to the performed surgical technique.³⁰ Recently, another RCT conducted to investigate the outcomes of MISTs with or without PRF for the treatment of isolated intrabony defects led to similar results with regard to the additional use of growth factors.³¹ Although the 36 defects treated in 36 patients showed comparable improvement in the clinical and radiographic parameters at a 6-month follow-up, the authors concluded that, due to the limitations of the study design, such as a small sample size and a short observation period, the effect of PRF could not be excluded.³¹

Discussion

Minimally invasive surgical techniques gave a new perspective with regard to the periodontal regeneration of isolated and multiple intrabony defects, aiming to reduce the invasiveness of the surgery and patient morbidity as well as chair time. The use of microsurgical instruments and high magnification⁴² as well as the introduction of the videoscope²⁵ made the single-flap MIST a feasible treatment modality in isolated 1-, 2- and 3-wall interproximal defects, which can be accessed and effectively debrided through a buccal or lingual flap window.^{17,19,24} Nevertheless, even with the aid of magnification, the tiny unilateral flap elevation often enough is not adequate to access defects that extend to the opposite side of the buccal or lingual flap.^{19,24} In such cases, the flap design has to be modified to the double-flap MIST with the elevation of the interdental papilla, and even vertical incisions for the adequate exposure of the defect.^{18,20,23} The double-flap MIST was considered suitable for the treatment of intrabony defects with pure 3-wall, or shallow 2- and/or 1-wall subcomponents.²³ Though, in cases when severe defects are extended in almost all the sides of the tooth and/or a deep buccal or lingual bone dehiscence is present, a larger flap reflection, and possibly vertical or periosteal incisions are demanded for the complete exposure of the defect and the application of the selected regenerative biomaterials.^{10,20} Thus, it seems that the extension of the defect determines the choice between the single- or double-flap MIST, and their limitations over deeper and more extensive defects.

The least possible increase in REC after the periodontal surgery of intraosseous defects is a great challenge for the operators aiming to fulfil the patients' demands with regard to esthetics, especially when the defect concerns the anterior esthetic zone. In the analyzed studies, the limited surgical trauma and the gentle manipulation of the soft tissues with MISTs led to the minimal,^{18,23} and even not significant recession of the gingival margin as compared to the baseline values.^{17,19,24} This is in accordance with the results of a meta-analysis by Clementini et al., demonstrating a mere and not statistically significant REC increase, using the MIST principles.⁴³ In a case series, in order to maintain the gingival margin stable after the surgical treatment of vertical defects, the authors applied atraumatic cause-related therapy, and subsequently the papilla preservation flap (PPF) technique in combination with the coronally advanced envelope-type buccal flap.⁴⁴ After 1 year, the shift of the gingival margin was not statistically neither clinically significant.⁴⁴ Therefore, both MISTs and the conventional PPF techniques can be considered as less impairing the patient's esthetics, and thus should be preferred over the traditional flap designs.⁴⁵ Nevertheless, there is a need to clarify if MISTs could demonstrate the same esthetic outcomes in all types of defects. Some studies indicated that neither the morphology of the defect^{17,19,46} nor the gingival biotype⁴⁶ could be considered as predictors for increased

recession, whenever MISTs were performed. In their study, Farina et al. concluded that the apical shift of the gingival margin after the periodontal surgery depended on the pre-surgical interproximal PD and suggested the additive use of biomaterials in deep defects located in esthetic areas.⁴⁶ In contrast, Cosyn et al. claimed that non-supportive defects and a thin-scalloped gingival biotype were related to a greater post-surgical increase in REC.²⁸ Thus, more studies are necessary to determine the risk factors that may compromise the patient's esthetics after periodontal surgical treatment.

It has been reported that the application of MISTs in the treatment of intrabony defects induces subtle postoperative pain and decreases patient morbidity.⁴³ The lesser invasiveness and the minimal surgical trauma caused by MISTs reduces the patient's discomfort and need for painkiller consumption, irrespective of the type of regenerative materials applied.¹⁰ Moreover, the perception of the post-surgical pain and the amount of analgesics taken by patients as well as the overall chair time have been found to be significantly lower for the MIST procedures as compared to the conventional surgical techniques.^{10,46} In addition to favorable patient-centered outcomes, a low percentage of postoperative adverse effects has been documented during the healing period.^{23,40,47} The primary wound closure was achieved in all cases examined, after the surgical procedure with MIST for the treatment of both single and multiple sites of defects. One week later, the rate of wound closure remained high at 95% and 100%, respectively.^{23,34} In their study, Trombelli et al. noted the absence of membrane exposure in the patients treated with bioresorbable membranes and bone graft, although 2 smokers were included.²⁴ This was another benefit of MISTs, which increased their efficacy in the treatment of intrabony defects, as membrane exposure has been proven to jeopardize the clinical outcomes of regenerative therapy.^{24,48} Mild postoperative pain and low patient morbidity rendered MIST a patient-friendly procedure and improved patient perception over surgical treatment.

The successful application of MISTs requires the use of magnification instruments for the adequate visualization of the surgical field.^{16,18,25} Although the operative microscope and the videoscope can serve this purpose better than loupes, their cost could be a major consideration for the clinicians.^{17,25} Moreover, since MISTs involve the surgical procedures performed under high magnification and the delicate manipulation of the soft tissues, they are regarded as clinically sensitive techniques, and require the clinicians' clinical skills and experience.

The defect anatomy is another factor that has to be further investigated in terms of its effect on the clinical efficacy of MISTs. In particular, intrabony defects with more residual bony walls are associated with a greater CAL gain and a decreased percentage of failure in clinical outcomes in case when MISTs are performed.^{28,49} Contrarily, in their study, Harrel et al. used MISTs with the aid of the videoscope and concluded that the number of residual bony walls

did not have a significant impact on CAL gain.¹⁷ Concerning the influence of the depth and the angle of the defect on the efficacy of MISTs, findings are also contradictory. Specifically, Cortellini et al. claimed that defects with a wider radiographic angle at baseline demonstrated less CAL gain, while the depth of the 3-wall subcomponent did not seem to affect clinical outcomes.³⁴ On the contrary, in another study by the same authors, the relation between the angle of the defect and CAL gain was found to be insignificant, while deeper 2- and 3-wall subcomponents showed more favorable clinical outcomes in terms of CAL gain.⁴⁹ Differences in the results of the abovementioned studies can be attributed to the small number of the participants. Thus, more studies with greater sample size are needed to clarify whether the defect anatomy can play a role as a determinant in the treatment of intrabony defects with MISTs.

Patient-centered factors, like poor oral hygiene and smoking, have been established to have a negative impact on the clinical outcomes of periodontal regeneration procedures.^{50,51} Although most of the studies in which MISTs were applied included patients with well-controlled full-mouth plaque and bleeding scores; the presence of plaque was related with a reduced CAL gain after the periodontal surgery treatment. In addition, the occurrence of post-operative complications and non-compliance with the scheduled recall sessions were also predictors for failure in CAL gain and vertical radiographic bone gain.^{27,28} It remains questionable if smoking can influence the clinical efficacy of MISTs. Trombelli et al. observed similar outcomes in terms of residual PD, CAL gain and REC increase between smokers and non-smokers, but significantly better early wound healing for the non-smokers group.⁵² Nevertheless, these clinical results seem to be inferior for heavy smokers (>10 cigarettes per day). The inclusion of light smokers and a small sample size can be considered as limitations of that study, and more investigations are needed for firm conclusions.

Over the last decades, the conventional surgical techniques in conjunction with various biomaterials have been widely used for the regeneration of bone defects that remain after the cause-related therapy, with significant benefits in terms of defect filling and periodontal attachment gain.^{13,53,54} Minimally invasive surgical techniques have been successfully combined with biologically active materials, such as amelogenins^{18,23,29,33–35,37} and growth factors,^{30,46} bone replacement grafts,^{27,28} and PRF,³¹ or in conjunction with two^{17,24,36,38,39,41} or more³⁶ different biomaterials for the periodontal regeneration of intrabony defects. Cortellini in 2012 proposed various regenerative strategies with MISTs depending on the defect morphology.²⁰ Thus, the single-flap MISTs can be applied in the case of any defect anatomy, either alone or combined with EMD. In addition, the double-flap MIST is suggested to be performed in conjunction with EMD for the treatment of self-supporting defects, and with EMD and graft for non-contained defects. Nevertheless, the additional use

of regenerative materials does not seem to significantly improve the clinical outcomes of MISTs, as reported in the available RCTs.^{30,31,36–38,41} In a meta-analysis of 4 RCTs, the mean differences between the MISTs + biomaterials and MISTs alone groups in terms of PD reduction, CAL gain and REC increase were 0.20 mm (95% confidence interval (CI): -0.26–0.66; $p = 0.40$), 0.24 mm (95% CI: -0.32–0.71; $p = 0.32$), and 0.03 mm (95% CI: -0.22–0.28; $p = 0.81$), respectively.⁵⁵ None of these differences was found to be statistically significant, confirming the fact that extra additives to MISTs do not provide further clinical benefits. On the contrary, the impact of biomaterials is important when they are combined with the conventional surgical techniques.¹³ Although, the PPF techniques improved the clinical efficacy of the conservative surgery in the treatment of intrabony defects,⁴⁵ periodontal regenerative therapy still leads to significantly superior clinical outcomes.¹³ The regenerative potential of MISTs as a stand-alone protocol in the treatment of intrabony defects may be a consequence of the primary wound closure, and the optimal wound and blood clot stability.^{18,23,24} In addition, the integrity of the interdental tissues can insure the necessary blood supply and space for the formation of the blood clot.^{19,24} These factors have been documented in experimental studies to be determinants for periodontal regeneration and the clinical efficacy of MISTs can be attributed to them.^{56–58} Though, the small sample sizes and the short follow-up periods of the available RCTs do not allow the extrapolation of definitive conclusions.

A practical question would be if the evolved flap design of MISTs could be considered more conducive in the surgical treatment of intrabony defects in comparison with the conventional PPF techniques. This was the main objective of 2 RCTs comparing the efficacy of the single-flap MISTs and of the double-flap PPF technique with or without the use of regenerative materials.^{46,59} In 2 groups of 14 patients, Trombelli et al. compared buccal MISTs and SPPT/MPPT for the treatment of intrabony defects.⁵⁹ Although the MISTs group demonstrated significantly greater PD reduction and CAL gain than the PPF group at a 6-month follow-up, these values were not statistically significantly different, when the comparison was adjusted to the pre-surgical PD and CAL values of the 2 groups. Both PD reduction and CAL gain were significantly different for the 2 groups as compared to baseline. An increase in REC was similar for the 2 groups at 6 months, but statistically significantly greater only for the MISTs group as compared to baseline. The authors had to take into account that the 2 groups were different in terms of age, number of smokers, PD, and bleeding score positive sites, though it was considered to have a significant impact only on PD reduction.⁵⁹ In another RCT, Schincaglia et al. assessed clinically and radiographically the efficacy of a single buccal or oral flap elevation MIST and the double-flap papilla preservation technique (SPPT, MPPT) combined with rhPDGF-BB and β -tricalcium phosphate (β -TCP) in

the treatment of 1-, 2- and 3-wall defects.⁶⁰ The 2 groups demonstrated significant changes in PD, CAL, REC, and the radiographic parameters at a 6-month follow-up, but the parameters were not significantly different between the 2 groups. Although PD reduction and CAL gain were slightly greater for the MISTs group, it included significantly fewer 1-wall defects than the PPF group. Nevertheless, patients from the MISTs group reported significantly lesser postoperative pain and painkiller consumption, while the early wound healing was better.⁶⁰ This was attributed to the lesser invasiveness and the limited flap extension of MISTs, which provide minimal trauma and better wound stability.⁴⁶ In a more recent RCT, Windisch et al. compared the clinical outcomes of MISTs + EMD with the more extended flap design of MPPT or SPPT + EMD for the treatment of 23 and 24 subjects, respectively.⁶¹ As in the previous two RCTs, no significant differences were observed in the clinical parameters between the 2 groups.⁶¹ It can be concluded that MISTs are at least as effective as the conventional PPF techniques, though this conclusion has to be further examined in more studies that will directly compare these techniques.

Conclusions

Minimally invasive surgical techniques significantly decreased surgical trauma in the soft tissues and the rates of wound failure after the periodontal surgery. As a consequence, many favorable clinical outcomes were observed in the studies in which MISTs were applied, while the patient perception of the periodontal surgery was improved. The adjunctive use of regenerative materials seems to have a less determinant effect on the clinical performance of MISTs. It is critical for the clinicians to estimate adequately the defect morphology in order to apply the most suitable surgical technique. Finally, more studies are required to evaluate the potential superiority of MISTs over the conventional techniques.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

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