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Original papers

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Reviews

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Assessment of dental anxiety levels among dental emergency patients during the COVID-19 pandemic through the Modified Dental Anxiety Scale

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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. Coronavirus disease 2019 (COVID-19) continues to affect dental emergency services worldwide. Dental anxiety (DA) is described as a common and distressing problem in terms of oral health maintenance.

Objectives. The present study aimed to evaluate DA levels as well as the COVID-19 fear and perception of control (COVID-19 FPC) in patients attending dental emergency clinics during the COVID-19 pandemic.

Material and methods. Sociodemographic, dental and medical data was obtained from the participants. A face-to-face questionnaire with questions referring to the reasons for the emergency dental visit, the visual pain scale, the Modified Dental Anxiety Scale (MDAS), and the COVID-19 Fear and Perception of Control Scale (COVID-19 FPCS) as well as additional questions concerning bruxism and a previous diagnosis of anxiety/panic attacks or depression was administered. The χ^2 test was used to analyze the data.

Results. A total of 1,439 patients were included in the study. The most common reason for the dental visit was pain (47.5%). The prevalence of DA was 5.1% (74/1,439). A significant association was found between DA and gender ($p = 0.020$). The incidence of severe pain was higher in patients with DA than in those without DA ($p = 0.002$). No significant differences in the MDAS scores were found between patients with and without a chronic disease ($p = 0.804$), with regard to the educational status ($p = 0.364$), or between the age groups ($p = 0.600$). The prevalence of a 'strongly agree' response to all questions in COVID-19 FPCS was higher in patients with DA as compared to those without DA.

Conclusions. Females and patients with severe pain were more likely to exhibit DA. In general, patients with DA strongly agreed with the statements of COVID-19 FPCS, which may indicate a correlation between the 2 scales.

Keywords: SARS-CoV-2, dental care, visual pain scale, coronavirus, Turkey, fear

Cite as
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Introduction

Fear is one of the emotions people often experience. It is a sudden involuntary behavior, an emotional reaction to a real or perceived threat.[1] Anxiety can be defined as a mood of uncertainty, a discomfort that can turn into panic or fear. Anxiety may lead to various physiological and behavioral disorders in individuals.[2]

One specific type of fear is that of the dentist, namely dentophobia, which occurs with the concern that dental treatment involves terrible experiences. If the fear of the dentist turns into anxiety, then patients may overreact during treatment. Moreover, this situation may cause patients to postpone their treatment, cancel appointments or attend follow-ups irregularly.[3] Dental fear and anxiety may develop during the appointment process, during the waiting period before treatment, and in relation to the dental instruments used and/or the procedures performed during treatment. The stimuli that typically cause the greatest fear and anxiety include seeing a dental injector, the injection procedure itself and the use of an aerator.[4] Fear and anxiety behaviors are considered as subjective reactions to pain.[5] On the other hand, anxiety can turn the pain into an unbearable feeling. Thus, it is necessary to understand the association between pain and anxiety. To meet this need, a scale was developed to measure dental anxiety (DA) in individuals. The Dental Anxiety Scale (DAS), which was created in 1978, serves this purpose.[6] In 2000, it was modified by adding the 'injection' criteria and called the Modified Dental Anxiety Scale (MDAS).[7] Today, MDAS is commonly used in research.[8–11] The prevalence of dental fear and anxiety across populations varies due to different measurement methods and patient groups. In the Turkish population, the prevalence has been found to be 21.3–23.5%.[12]

A novel type of coronavirus infection – coronavirus disease 2019 (COVID-19), which was first identified in Wuhan, China, in December 2019 and spread over the world – was declared a pandemic by the World Health Organization (WHO) in March 2020, with the first case detected in Turkey on March 11, 2020.[13] Societal fear, concern and anxiety levels increased at this time due to the uncertainty associated with the occurrence of the first case. The disease is transmitted through saliva, nasal drops, physical contact between individuals, and contact with contaminated surfaces.[14,15]

The bacterial and viral infection of patients and physicians has been a long-standing problem in dental clinics. However, with the COVID-19 pandemic, this problem has become a considerable danger.[16] In dental clinics, which belong to areas of the highest risk of the transmission and spread of the disease, the management of this problem has been attempted with the highest precaution and care, without suspending urgent healthcare services.

The characteristic difference between infectious diseases and other disorders is the presence of fear in the population. Fear is directly related to the infectiousness, severity and mortality risk of the disease.[17,18] Excessive fear can prevent people from thinking clearly and reasonably in their reactions to COVID-19. The current mitigation techniques for COVID-19 across the world are mainly focused on the control of the spread of the infection, the development of effective vaccines and the improvement of treatment. The psychosocial effects of the disease have not yet been adequately investigated.

If infection prevention protocols are not followed during dental treatment, then dental clinics can become a major source of the spread of COVID-19. Due to the high risk of contamination, non-urgent dental procedures have been temporarily postponed. Hence, it is of great importance to determine the incidence of dental visits, the reasons for visits and the amount of pain perceived by patients during the pandemic. Furthermore, changes in DA and fear levels in patients should be analyzed.

In this regard, the obtained data would enable us to understand the measures to be taken and the strategies to be followed during the pandemic, and to determine the profiles of dental clinic patients. The present study aimed to evaluate DA levels, and the COVID-19 fear and perception of control (FPC) in patients attending dental emergency clinics, and to assess the reasons for dental visits during the pandemic.

Material and methods

Following permission from the Turkish Ministry of Health (decision No. GO 20/547), the ethical approval of the study was obtained from the Clinical Research Ethics Committee at the Faculty of Medicine of Hacettepe University, Ankara, Turkey. This study was performed in compliance with the ethical principles of the Declaration of Helsinki, using the face-to-face interview method. Patients aged 18 years or older who reported to the Department of Dentomaxillofacial Radiology between June 2020 and September 2020 for emergency dental treatment, and who could speak, read and write in Turkish were included in this study. Written informed consent was obtained from all participants. Patients who did not give consent to participate were not included in the study. Incomplete questionnaire forms (i.e., unfilled or partially filled forms) was the sole exclusion criterion.

Sociodemographic, dental and medical data was obtained from the patients, and the assessment of pain levels was made using the visual pain scale. The patients were asked to rate their pain with a value between 1 and 10, while the participants without pain marked the option 'I have no pain'. The assessment criteria were as follows: 'no pain' – 0 points; 'worst pain imaginable' – 10 points. The classification for pain severity considered scores <3 as mild pain, scores 3–6 as moderate pain, and scores >6 as severe pain.[19] In addition, the patients were asked about

bruxism and a previous diagnosis of anxiety/panic attacks or depression. The reasons for the emergency dental visit were also recorded.

In the 2nd part of the questionnaire, a form including queries about COVID-19 FCP, generated by the authors, and the MDAS[7] form were administered. The validity and reliability of the Turkish version of MDAS had been previously examined by Ilgüy et al.[11] The scale consists of 5 questions. The minimum possible score for each question is 1 and the maximum score is 5. The adopted cut-off score on the scale was 19, and the participants who scored 19 or higher were considered to have high DA levels. In addition to these queries, the participants were asked to respond to the question "How do you feel about reporting to the dental clinic during the COVID-19 pandemic?" The COVID-19 Fear and Perception of Control Scale (COVID-19 FCPS) was generated after a detailed literature review.[20,21] It was mandatory to answer all of the questions in the questionnaire.

Statistical analysis

Frequency values expressed as number (n) and percentage (%) were used as descriptive statistics for the categorical variables. Pearson's χ^2 test was used to search for differences in terms of categorical variables, including demographic characteristics, and the medical and dental data. When the test result was statistically significant, pairwise comparisons were made using the appropriate χ^2 test with the Bonferroni correction.

The internal consistency of MDAS and COVID-19 FCPS was assessed via Cronbach's alpha. The internal consistency coefficient for MDAS was 88.3% and for COVID-19 FCPS it was 92.1%. Since there is no Turkish study on the validity and reliability of COVID-19 FCPS, each item was considered separately within the scope of this study, and the total score could not be obtained. The analyses were performed using Microsoft Word, v. 16.0 (Microsoft Corporation, Redmond, USA) and the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA). The significance level was set at 0.05.

Results

Overall, 1,644 patients attended the emergency clinic at the Department of Dentomaxillofacial Radiology within the chosen time period. Out of these, 205 patients decided not to participate in the study, and a total of 1,439 patients (595 males and 844 females) were included. The age of the patients ranged between 18 and 68 years, with a mean age of 34.8 ±14.2 years. The demographic characteristics of the sample are presented in Table 1. A total of 333 patients (23.1%) had 1 or more chronic diseases or other systemic conditions (Table 1). Hypertension (n = 83; 5.8%) was the most common disease followed by diabetes mellitus (n = 61; 4.2%) (Fig. 1).

Table 1. Demographic characteristics of the sample

Characteristics/Variables		*n* (%)
Gender	female	844 (58.7)
	male	595 (41.3)
Age [years]	18–25	408 (28.4)
	26–35	299 (20.8)
	36–45	281 (19.5)
	46–55	249 (17.3)
	≥56	202 (14.0)
Marital status	married	811 (56.4)
	single	628 (43.6)
Educational status	primary and secondary school graduate	381 (26.5)
	high school graduate	491 (34.1)
	undergraduate	137 (9.5)
	bachelor's degree	351 (24.4)
	postgraduate	79 (5.5)
Chronic disease	present	333 (23.1)
	absent	1,106 (76.9)
Previous diagnosis of anxiety/panic attacks or depression	present	134 (9.3)
	absent	1,305 (90.7)
Frequency of dental consulting	regularly	134 (9.3)
	occasionally	214 (14.9)
	due to a complaint	1,091 (75.8)
Bruxism	present	417 (29.0)
	absent	1,022 (71.0)

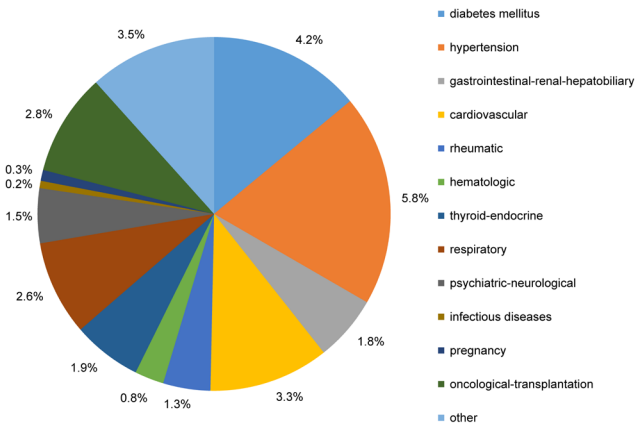

Fig. 1. Distribution of chronic diseases and other conditions among the participants

The percentages refer to the whole sample.

Previous diagnoses of anxiety/panic attacks or depression were more prevalent among females (n = 92; 10.9%) than males (n = 42; 7.1%) (p = 0.014). However, the prevalence rates were similar among the age groups (p = 0.365). Almost half of the patients with a previous diagnosis of anxiety/panic attacks or depression (65/134) reported an increase of varying degree in their symptoms during the pandemic.

One thousand and ninety-one patients (75.8%) stated that they reported to the dentist due to a complaint, with 214 (14.9%) visiting occasionally and 134 (9.3%) visiting regularly. The most common reasons for the emergency dental visit were toothache (47.5%) followed by tooth fracture (14.8%), abscess (13.7%) and tooth extraction (12.9%) (Fig. 2). Based on the scores obtained from the visual pain scale, more than half of the patients (n = 738; 51.3%) presented with severe pain, 364 (25.3%) with moderate pain, 77 (5.4%) with mild pain, and 260 (18.1%) with no pain.

The MDAS scores showed that 74 patients (5.1%) exhibited DA (MDAS ≥ 19). Dental anxiety was more common among females, the patients with severe pain, and those who felt very anxious or extremely anxious about visiting a dental clinic during the COVID-19 pandemic (p = 0.020, p = 0.002 and p < 0.001, respectively) (Table 2). No significant differences in DA were observed between the age groups, with regard to the educational status, and between patients with or without a chronic disease (p = 0.600, p = 0.364 and p = 0.804, respectively) (Table 2).

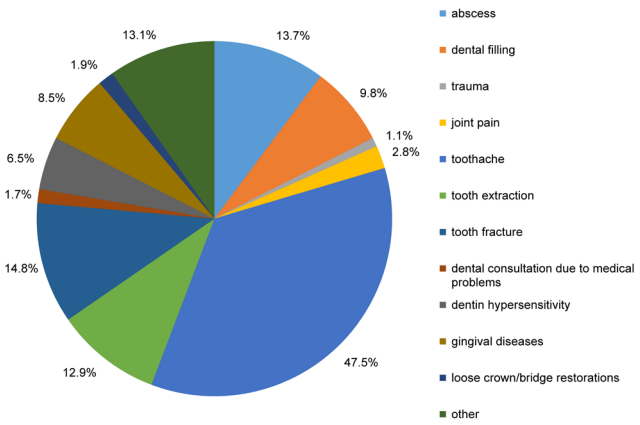

Fig. 2. Distribution of the reasons for the emergency dental visit
The percentages refer to the whole sample.

In total, 417 patients complained of bruxism. The reported bruxism was more common in females, the patients with a previous diagnosis of anxiety/panic attacks or depression, and the patients with higher MDAS scores (p < 0.001, p < 0.001 and p = 0.047, respectively) (Table 3). An association between the educational status and bruxism was also found (p = 0.010).

Table 2. Association between dental anxiety (DA) and gender, age, the educational status, chronic diseases, the visual pain scale scores, and feelings about reporting to the dental clinic during the coronavirus disease 2019 (COVID-19) pandemic

Variables		MDAS				p-value[c]
		without DA		with DA		
		n	%	n	%	
Gender	female	791	93.7	53	6.3[a]	0.020*
	male	574	96.5	21	3.5[a]	
Age [years]	18–25	388	95.1	20	4.9	0.600
	26–35	279	93.3	20	6.7	
	36–45	266	94.7	15	5.3	
	46–55	237	95.2	12	4.8	
	≥56	195	96.5	7	3.5	
Educational status	primary and secondary school graduate	357	93.7	24	6.3	0.364
	high school graduate	468	95.3	23	4.7	
	undergraduate	128	93.4	9	6.6	
	bachelor's degree	334	95.2	17	4.8	
	postgraduate	78	98.7	1	1.3	
Chronic disease	present	315	94.6	18	5.4	0.804
	absent	1,050	94.9	56	5.1	
Visual pain scale scores	0 (no pain)	257	18.8[a*]	3	4.1[b*]	0.002*
	<3 (mild)	74	5.4[a*]	3	4.1[a*]	
	3–6 (moderate)	348	25.5[a]	16	21.6[a]	
	>6 (severe)	686	50.3[a]	52	70.3[b]	
Response to: "How do you feel about reporting to the dental clinic during the COVID-19 pandemic?"	not anxious	350	25.6[a*]	2	2.7[a*]	<0.001*
	mildly anxious	558	40.9[a*]	8	10.8[b*]	
	considerably anxious	262	19.2[a*]	5	6.8[b*]	
	very anxious	116	8.5[a]	23	31.1[b]	
	extremely anxious	79	5.8[a]	36	48.6[b]	

MDAS – Modified Dental Anxiety Scale; [a, b] comparisons with the use of the Bonferroni correction at the level of p < 0.05; [a*, b*] 2 × 2 χ^2 tests with the Bonferroni correction at the level of p < 0.05; [c] Pearson's χ^2 test; * statistically significant.

The analysis of COVID-19 FCPS showed that the prevalence of a 'strongly agree' response to all questions was higher in patients with DA as compared to those without DA ($p < 0.001$) (Table 4).

Table 3. Association between the bruxism habit and gender, the educational status, a previous diagnosis of anxiety/panic attacks or depression, and dental anxiety (DA)

Variables		Bruxism		*p*-value[a]
		present	absent	
Gender	female	283 (33.5)	561 (66.5)	<0.001*
	male	134 (22.5)	461 (77.5)	
Educational status	primary and secondary school graduate	107 (28.1)	274 (71.9)	0.010*
	high school graduate	127 (25.9)	364 (74.1)	
	undergraduate	41 (29.9)	96 (70.1)	
	bachelor's degree	106 (30.2)	245 (69.8)	
	postgraduate	36 (45.6)	43 (54.4)	
Previous diagnosis of anxiety/panic attacks or depression	present	63 (47.0)	71 (53.0)	<0.001*
	absent	354 (27.1)	951 (72.9)	
MDAS	without DA	388 (28.4)	977 (71.6)	0.047*
	with DA	29 (39.2)	45 (60.8)	

Data presented as number (percentage) (*n* (%)). [a] Pearson's χ^2 test; * statistically significant.

Table 4. Assessment of the relationship between dental anxiety (DA) and responses to items in the COVID-19 fear and perception of control scale (COVID-19 FPCS)

Item	MDAS	I strongly disagree	I disagree	neutral	I agree	I strongly agree	*p*-value[a]
1. I am most afraid of COVID-19.	without DA	234 (17.1)	295 (21.6)	239 (17.5)	379 (27.8)	218 (16.0)	<0.001*
	with DA	4 (5.4**)	7 (9.5)	12 (16.2)	12 (16.2**)	39 (52.7**)	
2. It makes me uncomfortable to think about COVID-19.	without DA	212 (15.5)	279 (20.4)	176 (12.9)	533 (39.0)	165 (12.1)	<0.001*
	with DA	3 (4.1**)	8 (10.8)	5 (6.8)	25 (33.8)	33 (44.6**)	
3. My hands become clammy when I think about COVID-19.	without DA	429 (31.5)	535 (39.2)	185 (13.6)	159 (11.7)	56 (4.1)	<0.001*
	with DA	10 (13.5**)	21 (28.4)	17 (23.0**)	14 (18.9)	12 (16.2**)	
4. I am afraid of losing my life because of COVID-19.	without DA	269 (19.7)	312 (22.9)	179 (13.1)	420 (30.8)	185 (13.6)	<0.001*
	with DA	9 (12.2)	8 (10.8)	5 (6.8)	22 (29.7)	30 (40.5**)	
5. When watching news and stories about COVID-19 on social media, I become nervous or anxious.	without DA	240 (17.6)	320 (23.5)	197 (14.4)	488 (35.8)	119 (8.7)	<0.001*
	with DA	2 (2.7**)	6 (8.1**)	8 (10.8)	31 (41.9)	27 (36.5**)	
6. I cannot sleep because I am worrying about getting COVID-19.	without DA	568 (41.6)	519 (38.0)	128 (9.4)	115 (8.4)	35 (2.6)	<0.001*
	with DA	13 (17.6**)	24 (32.4)	17 (23.0**)	9 (12.2)	11 (14.9**)	
7. My heart races or palpitates when I think about getting COVID-19.	without DA	441 (32.3)	450 (33.0)	184 (13.5)	225 (16.5)	65 (4.8)	<0.001*
	with DA	15 (20.3**)	13 (17.6**)	15 (20.3)	15 (20.3)	16 (21.6**)	
8. COVID-19 is almost always terminal.	without DA	391 (28.7)	481 (35.3)	204 (15.0)	180 (13.2)	108 (7.9)	<0.001*
	with DA	14 (18.9)	18 (24.3)	19 (25.7**)	9 (12.2)	14 (18.9**)	
9. I am afraid of getting infected with COVID-19 from my circle or co-worker.	without DA	208 (15.2)	277 (20.3)	215 (15.8)	521 (38.2)	144 (10.5)	<0.001*
	with DA	5 (6.8)	6 (8.1)	7 (9.5)	32 (43.2)	24 (32.4**)	
10. I am afraid of getting the virus from my circle and carrying it to my family.	without DA	133 (9.7)	134 (9.8)	116 (8.5)	614 (45.0)	368 (27.0)	<0.001*
	with DA	3 (4.1)	4 (5.4)	2 (2.7)	27 (36.5)	38 (51.4**)	
11. I am afraid to talk to someone at close range.	without DA	136 (10.0)	185 (13.6)	198 (14.5)	628 (46.0)	218 (16.0)	<0.001*
	with DA	5 (6.8)	6 (8.1)	7 (9.5)	25 (33.8**)	31 (41.9**)	
12. The thought that I would be caught and quarantined with COVID-19 scares me.	without DA	189 (13.8)	253 (18.5)	197 (14.4)	509 (37.3)	217 (15.9)	<0.001*
	with DA	5 (6.8)	5 (6.8)	5 (6.8)	25 (33.8)	34 (45.9**)	
13. I am afraid to hear that people are dying because of COVID-19.	without DA	174 (12.7)	216 (15.8)	170 (12.5)	555 (40.7)	250 (18.3)	<0.001*
	with DA	4 (5.4)	2 (2.7**)	6 (8.1)	29 (39.2)	33 (44.6**)	

Data presented as *n* (%). [a] Pearson's χ^2 test; * statistically significant; ** significance as a result of the comparison following the Bonferroni correction at the level of $p < 0.05$ (when the comparison test assumptions were provided, the significance of the difference between the 2 percentages was obtained with the test, and otherwise with the help of the χ^2 tests).

Discussion

The present study was mainly designed to determine the reasons for attending dental emergency clinics during the COVID-19 pandemic, and to assess DA levels, as measured with MDAS, with regard to the sociodemographic characteristics and COVID-19 FCP of the patients, with the latter measured with COVID-19 FCPS.

The majority of the patients in this study had irregular dental care habits, as they reported to the dental clinic only due to a complaint. Most of them presented with severe pain and the primary reason for seeking emergency care was toothache. These results confirm that pain relief is by far the main reason for attending dental emergency clinics.[22–24] The prevalence of DA in the present study (5.1%) was slightly lower than in previous studies (8–11.6%), as measured by means of MDAS (cut-off score ≥19).[8–11] Based on the present findings, DA was more common in females, which is in accordance with the results of previous research.[25–30] In a previous study, it was stated that females exhibited higher levels of anxiety, because, in comparison with males, they perceived outbreaks as more dangerous.[31] Therefore, performing the present study during the COVID-19 pandemic may also have resulted in higher DA levels in females. However, there have also been studies that did not found any association between gender and DA.[32–36]

Our results showing no significant association between age and DA are also consistent with previous reports.[25,32,36] This finding might be attributed to the fact that older patients may report to the emergency clinics less often, as they may have fewer or no teeth.[32] Another possible reason may be the restrictions imposed on patients aged 55 years or older, such as a national lockdown, as in this study. However, conflicting results have also been reported.[7,37–39] Consistent with several previous reports,[32,33,36,38–41] no association was found between the educational status and the MDAS scores in the present study. This might be due to the small number of patients with higher educational levels in the study group.[32] However, there are also other reports in the literature that present different results.[28,42,43] A possible explanation for these latter observations is that a high educational level may be associated with better oral health and regular dental check-ups, which may support a decrease in DA as the educational level increases.[43]

The present study found an association between the reported bruxism and the MDAS scores, consistent with the results of previous studies.[44–46] This might be due to the fact that bruxism is a reflection of the individual's response to stress in the oral cavity.[44] It has also been suggested that the pain experiences of individuals may impact their DA levels.[27] In the present study, an association was established between severe pain and DA, a finding consistent with the literature.[47,48] We found responses of 'very anxious' and 'extremely anxious', related to the respondents' feelings about visiting dental clinics during the COVID-19 pandemic, correlated with DA. This result is not surprising, considering the fact that people who encounter such an extraordinary situation for the first time may express emotions such as fear, anxiety or stress. Another reason is that COVID-19 causes not only physical health problems, but also a series of psychosocial disorders.[49]

The COVID-19 FCPS used in the present study was prepared specifically for the period of the pandemic following a review of the literature. The authors believe that there may be correlations between the scale and MDAS in many aspects. It has been shown that most individuals are afraid of being infected with the virus in crowded environments and transmitting it to their families/relatives.[50] Therefore, it may be suggested that being together with other patients in the waiting rooms of dental clinics and the fact that the patient has to remove their mask during treatment/examination may increase the level of anxiety. This may also explain why patients with DA strongly agreed with the statements of COVID-19 FPCS in the present study.

Limitations

This study has some limitations. First, the study was conducted in a dental emergency clinic in Ankara, which may potentially limit the generalizability elsewhere in Turkey, especially in terms of the demographic characteristics of the patient population. Another limitation is that the data presented in this study is self-reported, and partly dependent on the participants' honesty and recall ability. Thus, the data may be subject to recall bias. The COVID-19 FPCS generated for the present study has not been checked for validity and reliability. Therefore, the items of the scale were analyzed separately. Finally, as a result of the cross-sectional nature of this study, the assessment of DA levels in the patients was made at a single time point.

Conclusions

With the COVID-19 pandemic, the whole world came up against an unexpected danger. Uncertainty regarding the infectiousness and virulence of the virus may have led to changes in the anxiety levels of individuals. The prevalence of DA in the present study was slightly lower as compared to previous reports in the literature. Nevertheless, females and patients with severe pain were found to exhibit increased DA. In general, patients with DA strongly agreed with the statements of COVID-19 FPCS, which may indicate a correlation between the 2 scales.

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Anxiety and harmful oral habits in preschool children during the 2020 first-wave COVID-19 lockdown in Turkey

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Abstract

Background. Due to curfew and quarantine practices designed to reduce the spread of coronavirus disease 2019 (COVID-19), social isolation has tested the psychological limits of children.

Objectives. The authors evaluated parent-observed symptoms of anxiety in preschool children with harmful oral habits during the curfew period in Turkey.

Material and methods. The authors prepared a questionnaire with Google Forms that was distributed through social media applications (e.g., WhatsApp) to 405 parents recruited via snowball sampling. To measure children's symptoms and levels of anxiety, the Spence Preschool Anxiety Scale (SPAS) was used. Harmful oral habits that might develop in children during the curfew were investigated.

Results. Separation anxiety and physical injury anxiety were reported by the parents more frequently than general anxiety and obsessive-compulsive disorder. Also, the presence of tantrums ($p = 0.010$), crying attacks ($p = 0.010$) and aggression ($p = 0.010$) were reported by the parents in these children. It was observed that the habits of finger sucking ($p = 0.010$), nail biting ($p = 0.040$) and lip biting ($p = 0.010$) that were present before the curfew decreased significantly after the curfew.

Conclusions. Children aged 3–7 years can develop anxiety about physical injuries and about being separated from their parents as well as tantrums and crying attacks. Their harmful oral habits (i.e., finger sucking, nail biting and lip biting) all decreased during the curfew period.

Keywords: children, aggression, finger sucking, nail biting, separation anxiety disorder

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Introduction

Coronaviruses (CoV) are a family of viruses that can cause illnesses ranging from severe respiratory infections to mild conditions, like the common cold. In January 2020, a novel coronavirus, 2019-nCoV, began causing cases of pneumonia in Wuhan, China.[1] The virus was similar to severe acute respiratory syndrome coronavirus (SARS-CoV), but had never been detected in humans before.

On March 11, 2020, following the spread of coronavirus disease 2019 (COVID-19) in 113 countries, including Turkey, the World Health Organization (WHO) declared COVID-19 a pandemic. Since the first infection with the virus diagnosed in humans until August 16, 2020, approx. 21.2 million cases and 761,000 deaths were reported worldwide.[1] For those and other reasons, COVID-19 has been perceived as a major threat not only to public health, but also to the global economy.

Health authorities in Turkey and other countries have advised canceling conferences, ceremonies, and various art and cultural events, suspending educational activities, imposing bans on domestic and international air travelling, and initiating quarantine practices to reduce the spread of the virus. In Turkey, to mitigate the risks posed to public health by curbing people's interpersonal contact and social mobility, the government implemented a curfew prohibiting children aged <20 years from being in public places between April 3, 2020 and June 1, 2020.[2] Such practices to control the spread of COVID-19 in Turkey significantly altered daily routines by requiring long-term periods spent at home, eliminating direct contact with friends and, in turn, causing boredom, and even familial behavioral problems, which psychologically challenge children as much as adults.[2–5] Regarding children in particular, studies have shown that those who are more stressed move less, spend more time in front of the television or computer, and consume less healthy foods, especially on weekends or during summer holidays, when not attending school.[2,6,7] Beyond that, their fear of asking questions about the pandemic and the health of relatives, poor sleep, including nightmares, poor appetite, physical discomfort, agitation, inattention, and separation problems may prompt the development of psychological disorders in children.[3] Such disorders in children may promote harmful oral habits or change the existing good habits.[5,8–10]

The authors aimed to evaluate the symptoms of anxiety observed by parents in their 3–7-year-old children, and assess the children's harmful oral habits before and after the 2 months of Turkey's curfew.

Subjects and methods

This study received ethical approval from the Clinical Research Ethics Committee at the Faculty of Medicine of Erciyes University, Kayseri, Turkey (No. 352-2020).

Setting and participants

To prevent the spread of COVID-19 and avoid overburdening the healthcare system, the Turkish government has promoted the reduction of face-to-face interviews since spring 2020. For that reason, the consent form and the questionnaire, developed in compliance with the Declaration of Helsinki, were created with Google Forms and distributed through social media applications (e.g., WhatsApp) to parents recruited via snowball sampling by providing access to https://docs.google.com/forms/d/12n1QnyRH9LW6wpfrFtN7jscb2m1rkKDjoVdIU5mW8Hc/edit. A total of 783 questionnaires were completed; however, 147 forms were excluded because of children and parents having mental and systemic diseases. Also, 231 forms were excluded due to children who were over 7 years old. Ultimately, 405 questionnaires were included in this study.

Questionnaire content

The 1st questionnaire was sent to the participants in the 1st week of April 2020 to evaluate harmful oral habits, and the 2nd questionnaire was sent to the same e-mail addresses in the 2nd week of June 2020 to re-evaluate harmful oral habits, determine the Spence Preschool Anxiety Scale (SPAS) scores and assess different behavioral problems.

The questionnaire consisted of 5 parts.

The 1st part asked the participating parents questions about their own and their children's demographic information, including their age, gender, level of education, their children's age, and family structure (e.g., the number of children).

The 2nd part of the questionnaire incorporated SPAS. The parents answered questions listed under "General anxiety", "Obsessive-compulsive disorder", "Separation anxiety", and "Physical injury anxiety" on a 5-point Likert scale from 0 ('not true at all') to 4 ('very often true'). However, the parents did not answer questions listed under "Social anxiety" due to their irrelevance amid the curfew.

The 3rd part asked the parents questions about different symptoms of anxiety observed in their children, namely tantrums, crying attacks and aggression, to be answered with either 0 (absent) or 1 (present). The authors sought a yes or no answer to whether behavioral symptoms developed during the period from the beginning to the end of the curfew.

The 4th part asked whether the children demonstrated any harmful oral habits, namely finger sucking, nail biting and lip biting, before the curfew. A 4-point scale referring to the frequency of harmful habit episodes (0 = 'none'; 1 = 'few'; 2 = 'some'; 3 = 'many') was used.

In a follow-up, using the same scale, the 5th and final part of the questionnaire asked whether the children demonstrated the same habits after the curfew had ended.

Statistical analysis

The authors analyzed the data with the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA). In particular, the authors used the independent samples *t* test to compare the anxiety scores according to different symptoms of anxiety, the McNemar–Bowker test to examine any changes in the frequency of harmful oral habits, and the *t* test to examine changes in the presence of harmful oral habits before and after the curfew. The one-sample *t* test analysis was carried out to analyze differences between the anxiety levels obtained in the present study and the mean scores reported in the norm study.[11] The post hoc Šídák pairwise comparison test was performed to determine whether the values obtained for the anxiety subdimensions in the present study were statistically significantly different or not, and to rank the values of the anxiety subdimensions. Categorical data was recorded as number (*n*) and percentage (%), and quantitative data as mean (*M*) and standard deviation (*SD*). Any *p*-value <0.05 was considered to indicate statistical significance.

Table 1. Demographic data of the respondents

Demographic data		*M* ±*SD* 95% *CI* (min–max) or *n* (%)
Parent's age [years]		33.8 ±4.8 33.0 (20.0–45.0)
Children's age [years]		5.1 ±1.2 5.0 (0.6–7.0)
Parent's gender	female	376 (92.8)
	male	29 (7.2)
What is your educational background?	university	251 (62.0)
	high school	80 (19.8)
	doctorate/expertise	40 (9.9)
	other	34 (8.4)
Does your child go to preschool?	yes	338 (83.5)
	no	67 (16.5)
Does your child have a sibling?	yes	277 (68.4)
	no	128 (31.6)
Family type	nuclear	366 (90.4)
	extended	39 (9.6)

M – mean; *SD* – standard deviation; *CI* – confidence interval; min – minimum; max – maximum; n – number; % – percentage.

Results

There were 405 parents who responded to the questionnaire and were included in the analysis. Their mean age was 33.8 years, and by gender, 92.8% were women and 7.2% were men. By level of education, 19.8% of the respondents had graduated from high school, 62.0% from university and 9.9% from doctoral studies. The children's mean age was 5.1 years. The children of a significant majority of the respondents (83.5%) were attending preschool and the children of as many as 68.4% of the respondents had at least 1 sibling (Table 1).

As mentioned, the questions on SPAS address 5 types of anxiety: general anxiety (i.e., Q1, Q4, Q8, Q14, and Q28); obsessive-compulsive disorder (i.e., Q3, Q9, Q18, Q21, and Q27); separation anxiety (i.e., Q6, Q12, Q16, Q22, and Q25); physical injury anxiety (i.e., Q7, Q10, Q13, Q17, Q20, Q24, and Q26); and social anxiety (i.e., Q2, Q5, Q11, Q15, Q19, and Q23). The questions listed under "Social anxiety" were not answered due to their irrelevance amid the curfew. Along those lines, separation anxiety and physical injury anxiety were reported relatively often among the children (Table 2).

It was found that there was a significant difference between the general anxiety levels of the participants in the present study and those obtained in the norm study, with the latter ones being higher ($p = 0.010$). It was found that there was a significant difference between the obsessive-compulsive disorder levels of the participants in the present study and those obtained in the norm study, with the latter ones again being higher ($p = 0.010$). It was observed that there was a significant difference between the physical injury anxiety levels of the participants in the current study and those obtained in the norm study, which were lower in this case ($p = 0.010$). Finally, it was observed that there was a significant difference between the separation anxiety levels of the participants in the current study and those obtained in the norm study, which were again lower ($p = 0.010$) (Table 3).

It was found that the mean scores for general anxiety, obsessive-compulsive disorder, physical injury anxiety, and separation anxiety were significantly different ($p = 0.010$); it was due to the higher levels of physical injury anxiety and separation anxiety as compared to other anxiety subdimensions ($p = 0.010$; $p < 0.05$) (Table 4).

Regarding the examined symptoms of anxiety (i.e., tantrums, crying attacks and aggression), the frequency of tantrums was 28.6%, of crying attacks – 32.8% and of aggression (i.e., self-hitting and hitting others) – 26.7% (Table 5). The children with higher SPAS scores had more tantrums ($p = 0.010$). The same correlation occurred in the case of crying attacks ($p = 0.010$) and aggression ($p = 0.010$) (Table 6).

It was observed that the finger sucking habit observed in the children before the curfew decreased significantly after the curfew ($p = 0.010$). Similarly, significant decreases where observed after the curfew in the nail biting habit ($p = 0.040$) and in the lip biting habit ($p = 0.010$) as compared to the situation before the curfew (Table 7). In particular, 99.2% of the children did not suck their fingers before the curfew, nor did they afterward, whereas 66.7% with few finger sucking episodes before the curfew kept their habit at the same level afteward.

Table 2. Spence Preschool Anxiety Scale (SPAS) scores

Anxiety subdimension	SPAS questions	SPAS answers				
		not true at all	seldom true	sometimes true	quite often true	very often true
General anxiety	Q1	112 (27.7)	120 (29.6)	100 (24.7)	59 (14.6)	14 (3.5)
	Q4	124 (30.6)	148 (36.5)	69 (17.0)	48 (11.9)	16 (4.0)
	Q8	166 (41.0)	122 (30.1)	58 (14.3)	41 (10.1)	18 (4.4)
	Q14	264 (65.2)	85 (21.0)	32 (7.9)	9 (2.2)	15 (3.7)
	Q28	246 (60.7)	85 (21.0)	38 (9.4)	21 (5.2)	15 (3.7)
Obsessive-compulsive disorder	Q3	178 (44.0)	107 (26.4)	61 (15.1)	37 (9.1)	22 (5.4)
	Q9	186 (45.9)	90 (22.2)	55 (13.6)	34 (8.4)	40 (9.9)
	Q18	221 (54.6)	82 (20.2)	48 (11.9)	22 (5.4)	32 (7.9)
	Q21	223 (55.1)	97 (24.0)	43 (10.6)	27 (6.7)	15 (3.7)
	Q27	293 (72.3)	53 (13.1)	29 (7.2)	11 (2.7)	19 (4.7)
Separation anxiety	Q6	81 (20.0)	73 (18.0)	56 (13.8)	87 (21.5)	108 (26.7)
	Q12	115 (28.4)	112 (27.7)	85 (21.0)	44 (10.9)	49 (12.1)
	Q16	173 (42.7)	127 (31.4)	43 (10.6)	24 (5.9)	38 (9.4)
	Q22	186 (45.9)	99 (24.4)	44 (10.8)	36 (8.8)	40 (9.8)
	Q25	235 (58.0)	91 (22.5)	43 (10.6)	26 (6.4)	10 (2.5)
Physical injury anxiety	Q7	134 (33.1)	92 (22.7)	69 (17.0)	50 (12.3)	60 (14.8)
	Q10	220 (54.3)	86 (21.2)	49 (12.1)	28 (6.9)	22 (5.4)
	Q13	122 (30.1)	130 (32.1)	63 (15.6)	51 (12.6)	39 (9.6)
	Q17	152 (37.5)	80 (19.8)	71 (17.5)	53 (13.1)	49 (12.1)
	Q20	109 (26.9)	108 (26.7)	76 (18.8)	49 (12.1)	63 (15.6)
	Q24	205 (50.6)	96 (23.7)	45 (11.1)	34 (8.4)	25 (6.2)
	Q26	80 (19.8)	126 (31.1)	70 (17.3)	61 (15.1)	68 (16.8)
Social anxiety	Q2	–	–	–	–	–
	Q5	–	–	–	–	–
	Q11	–	–	–	–	–
	Q15	–	–	–	–	–
	Q19	–	–	–	–	–
	Q23	–	–	–	–	–

Table 3. Evaluation of the anxiety subdimensions with regard to the norm study[11]

Anxiety subdimension	Present study ($N = 405$)	Norm study ($N = 461$)	p-value†
General anxiety	10.10 ±3.92[a]	12.60 ±3.23[b]	0.010*
Obsessive-compulsive disorder	9.54 ±4.33[a]	14.93 ±3.67[b]	0.010*
Physical injury anxiety	16.20 ±5.37[b]	9.61 ±3.17[a]	0.010*
Separation anxiety	17.92 ±4.41[b]	12.72 ±4.07[a]	0.010*
Total SPAS score	53.76 ±13.18[b]	49.86 ±12.01[a]	0.020*

Data presented as $M \pm SD$. SPAS – Spence Preschool Anxiety Scale; † one-sample t test; * statistically significant (a < b).

Table 4. Analysis of differences between the anxiety subdimensions in terms of the Spence Preschool Anxiety Scale (SPAS) scores

Anxiety subdimension	$M \pm SD$	p-value†	Post hoc test result
General anxiety	10.10 ±3.92	0.010*	3.4 > 1.2 ($p < 0.05$*)
Obsessive-compulsive disorder	9.54 ±4.33		
Physical injury anxiety	16.20 ±5.37		
Separation anxiety	17.92 ±4.41		

† post hoc Šídák pairwise comparison test; * statistically significant.

Table 5. Frequency of behavioral problems

Behavioral problem	Absent	Present
Tantrums	289 (71.4)	116 (28.6)
Crying attacks	272 (67.2)	133 (32.8)
Aggression	297 (73.3)	108 (26.7)

Data presented as n (%).

Table 6. Presence of behavioral problems according to the Spence Preschool Anxiety Scale (SPAS) scores

Behavioral problem	Absent	Present	p-value†
Tantrums	52.27 ±16.29	57.11 ±17.75	0.010*
Crying attacks	52.68 ±13.49	55.82 ±17.46	0.010*
Aggression	51.97 ±15.89	58.63 ±16.57	0.010*

Data presented as $M \pm SD$. † independent samples t test; * statistically significant.

By contrast, 33.3% of the same group stopped finger sucking altogether. While 28.6% of the children who were observed to suck their fingers moderately before the curfew stopped finger sucking, 28.6% of them continued to suck a little and 42.9% of them did not alter the frequency of the habit. Trends in nail biting, however, differed from those in finger sucking. While 23.9% of the children who bit their nails before the curfew stopped biting their nails afterward, 71.6% continued to bite their nails, but not often, and 3.0% were reported to bite their nails at a moderate frequency. Finally, lip biting also differed after vs. before social isolation (Table 8).

Discussion

Staying at home can facilitate the improvement of social relations between family members, and such close relationships between children and the rest of the family can prevent physical and psychological problems that may otherwise develop.[12] However, owing to a prevalent life-threatening illness, children's levels of anxiety and stress while at home may rise.[8] Anxiety is defined as a feeling of powerlessness, fear and discontent in a seemingly threatening environment.[13] If children's anxiety can be appropriately controlled by their families, then they can overcome the condition of distress and become stable, both emotionally and physiologically, even in social isolation during a pandemic such as COVID-19.[14] Considering that possibility, the authors evaluated the anxiety observed by parents in their 3–7-year-old children in social isolation during Turkey's 2-month curfew. To that purpose, the authors used SPAS, which has been validated for Turkish children and the reliability of which has been confirmed.[11,15] The questionnaire was administered using the snowball technique through WhatsApp and other social media applications. The questionnaire addressed 5 types of anxiety on a Likert-type scale, but social anxiety questions were not included. The reason why the social anxiety questions were not answered is the "stay at home" and "curfew restriction under the age of 20" practices. Therefore, it was not possible to statistically compare the before and after scores on the same SPAS questionnaire. Hence, the authors compared the SPAS anxiety scores obtained in the present study with the norm values specified for Turkish preschool children.[11] In the present study, although the general anxiety and obsessive-compulsive disorder data was found to be lower than the norm values, the total SPAS anxiety scores were found to be higher than in the norm study. This difference was due to the higher levels of separation anxiety and physical injury anxiety observed in the present study.

Earlier research, which did not indicate the age range, found that quarantine practices could lead to the development of separation anxiety as well as a fear of infection in children, which in the long term might affect a child's mental health.[4] Added to that, Spence et al. posited that

Table 7. Changes in the presence of harmful oral habits before and after the curfew

Harmful oral habit	Evaluation time	Absent	*p*-value†
Finger sucking	before the curfew	0.14 ±0.51	0.010*
	after the curfew	0.10 ±0.41	
Nail biting	before the curfew	0.42 ±0.78	0.040*
	after the curfew	0.38 ±0.77	
Lip biting	before the curfew	0.19 ±0.53	0.010*
	after the curfew	0.15 ±0.48	
Total	before the curfew	0.76 ±1.28	0.010*
	after the curfew	0.62 ±0.74	

Data presented as *M* ±*SD*. † *t* test; * statistically significant.

Table 8. Changes in the frequency of harmful oral habits before and after the curfew (McNemar–Bowker test)

Harmful oral habit	Before the curfew	After the curfew			
		none	few	some	many
Finger sucking	none	368 (99.2)	2 (0.5)	0 (0.0)	1 (0.3)
	few	5 (33.3)	10 (66.7)	0 (0.0)	0 (0.0)
	some	4 (28.6)	4 (28.6)	6 (42.9)	0 (0.0)
	many	0 (0.0)	2 (40.0)	1 (20.0)	2 (40.0)
Nail biting	none	284 (96.3)	11 (3.7)	0 (0.0)	0 (0.0)
	few	16 (23.9)	48 (71.6)	2 (3.0)	1 (1.5)
	some	4 (14.8)	4 (14.8)	14 (51.9)	5 (18.5)
	many	2 (12.5)	0 (0.0)	0 (0.0)	14 (87.5)
Lip biting	none	345 (98.9)	3 (0.9)	0 (0.0)	1 (0.3)
	few	20 (45.5)	23 (52.3)	1 (2.3)	0 (0.0)
	some	1 (16.7)	1 (16.7)	3 (50.0)	1 (16.7)
	many	1 (16.7)	0 (0.0)	2 (33.3)	3 (50.0)

Data presented as *n* (%).

separation anxiety and physical injury anxiety were common in preschool children,[16] while Edwards et al. observed children's fear of physical injury marked by a fear of darkness, disease and specific animals.[17] Consistent with those results, the authors of the present study reported that separation anxiety and physical injury anxiety were the most common trends in the respondents' children aged 3–7 years. Consequently, communication among family members is pivotal in controlling not only the spread of the infection, but also separation anxiety.[8] In communication with children aged 3–7 years, it is necessary to use concrete language in order to avoid misunderstanding.

In research on the emotional and behavioral changes in children during COVID-19, Jiao et al. found that anxiety increased in children aged 3 months–17 years during the COVID-19 pandemic,[18] while Pisano et al. observed that children aged 4–10 years in the same situation had trouble sleeping and exhibited sudden emotional changes.[19] In the present study, the authors also investigated behavioral problems, such as tantrums, crying attacks and aggression, among children during Turkey's curfew. Since behaviors are generally acquired from what individuals observe in their environments or during social learning, behavioral problems can occur in preschool children due to the effects of environmental factors.[20] Examinations of children aged 3–7 years and adolescents in groups have revealed that parental attitudes and behaviors appear to be important factors in determining children's and adolescents' positive social behaviors.[21] The results of this study showed that the frequency of tantrums, crying attacks and aggressive behaviors increased significantly after the curfew. The authors of the present study believe that increases in separation anxiety and physical injury anxiety might have increased the frequency of tantrums and crying attacks. In the current study, the frequency of finger sucking, lip biting and nail biting decreased significantly during the curfew. In general, anxiety, fear, tension, and jealousy can damage children's sense of trust in their parents, and prompt finger sucking, nail biting and lip biting due to the feeling of deprivation.[22] These parafunctional oral habits are harmful, as they can directly affect the growth and development of the oral and maxillofacial skeleton. In addition, malocclusion can occur.[10,23,24] Although research on children with anxiety is likely to report harmful oral habits,[25,26] this study found that such harmful oral habits as finger sucking, lip biting and nail biting decreased significantly during the curfew. The occurrence of harmful oral habits may originate as an emotional need.[27] Although the authors do not know how children spent time with their parents during social isolation, the reason for decreases in harmful oral habits might be a sense of trust between parents and children. Spending time together helps to develop a sense of trust in the family, which may prevent harmful oral habits, and thereby prevent malocclusion.[28]

Limitations

One limitation of this study is that the SPAS scores at the beginning of the social isolation period could not be obtained, as the social anxiety questions had not been answered by parents due to the curfew. The study aimed to investigate the effects of social isolation on children's harmful oral habits, and their anxiety levels and symptoms instead of explaining the reasons for the related changes. Further studies are warranted to evaluate the amount of screen time or time spent on other activities by children during isolation, alone or with their parents, to clarify the possible reasons for changes in oral habits as well as anxiety levels and symptoms.

Conclusions

Children aged 3–7 years who cannot attend school, or sufficiently participate in social and physical activities can develop anxiety about suffering physical injuries and becoming separated from their parents. At the same time, this study showed that tantrums and crying attacks increased, while harmful oral habits (i.e., finger sucking, nail biting and lip biting) all decreased in children during Turkey's curfew.

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Craniofacial trauma in Brazilian child victims of traffic accidents: A single-trauma center analysis

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Abstract

Background. Road traffic accidents are a significant public health problem and a major cause of economic loss globally.

Objectives. The aim of the study was to describe the epidemiological profile of child victims of traffic accidents and the prevalence of craniofacial trauma in a municipality in the northeastern region of Brazil.

Material and methods. A documentary study was carried out through the analysis of the medical records of the traffic accident victims hospitalized in emergency wards in the municipality of Campina Grande, Paraíba, Brazil, from January to December 2016. A total of 1,884 medical records were evaluated, among which 85 (4.5%) referred to children aged 0–12 years. The information related to the sociodemographic profile of the victims, traffic accidents and clinical variables was collected. The data was analyzed using descriptive and inferential statistics, and a 5% level of significance was adopted.

Results. The victims were predominantly males (64.7%) and 9–12-year-old children (43.5%). Most accidents were recorded in the evening (48.2%) and on the weekend (32.9%). Among the victims, 37.6% were involved in motorcycle accidents. Craniofacial injuries were found in 44.7% of the children. Most victims were not admitted to the intensive care unit (ICU) (80.0%). Craniofacial trauma was significantly associated with motorcycle ($p = 0.043$) and automobile accidents ($p = 0.013$).

Conclusions. Our findings suggest that motorcycles are the vehicles most frequently involved in traffic accidents, which predominantly affect males and children aged 9–12 years, and result in a high prevalence of craniofacial trauma.

Keywords: epidemiology, child, maxillofacial injuries, motorcycles

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Introduction

Throughout the world, many families are devastated by the loss of a child due to an unintentional injury, which makes these deaths a serious public health problem.[1] Unintentional injuries are those caused by traffic accidents, drowning, falls, burns, and poisoning.[1] According to the Global Burden of Disease Study,[2] the global prevalence of injuries in transport accidents was approx. 241 million individuals in 2016, and studies have shown that these accidents represent the main etiology of craniofacial trauma among children.[3,4]

The consequences of trauma in the craniofacial region can include any combination of bone, soft tissue or dental injury.[5] The location and pattern of the injury depend on the interrelation between the etiology of the injury and the magnitude of the impact as well as on the characteristics of the victim's developmental stage.[6] Bone flexibility, the presence of dental germs, the smaller size of the paranasal sinuses, the greater cranium/face ratio, and the thick buccal fat pad make children more likely to endure craniofacial trauma.[7] A mechanism of injury involving greater impact, such as a traffic accident, is required for this trauma to occur.[4]

Due to differences in anatomical development, craniofacial trauma can be considered uncommon in children when compared to adults,[8] but facial injuries to soft tissues occur more frequently in children.[7,9] Studies indicate that there is a general increase with age in the incidence of facial trauma and a decrease in the incidence of head trauma.[8] However, some studies have reported an increasing incidence of craniofacial trauma with increasing age.[3,5]

In a retrospective cross-sectional study carried out in the United Arab Emirates, it was observed that from a total of 475 craniofacial injuries, 6.5% occurred in the 1st decade of the patient's life and 18.4% in the 2nd one.[5] A retrospective cohort study carried out in the United States that used the 2012 Kids' Inpatient Database found that out of 20,070 cases of craniofacial trauma, 35.4% occurred between 0 and 10 years of age, and 63.7% between 11 and 20 years of age.[3] Transport accidents were the main etiological factor.[3] A cross-sectional survey carried out in the state of Paraíba, Brazil, revealed that 14.4% of the victims of transport accidents were children and adolescents, with head trauma being 5 times more frequent in the age group 0–4 years as compared to children aged 5–9 years.[10]

Craniofacial injuries in the pediatric population can have negative esthetic and functional effects, causing disfigurement, morbidity, and potential growth and development disorders. Moreover, they cause a great economic burden for families and health systems.[3,6] Therefore, the need to characterize the profile of the victims of craniofacial injuries from this population group and to analyze the different causes of these injuries makes this study relevant. Such knowledge would allow the planning of efficient first aid to obtain relevant recovery, the evaluation of the distribution of work teams, the determination of the related public policies, and assistance in calculating costs for health services.[11] Considering these factors, the aim of this study was to describe the epidemiological profile of child victims of traffic accidents and the prevalence of craniofacial injuries in a municipality in the northeastern region of Brazil.

Material and methods

Ethical statement

This study was approved by the Research Ethics Committee of the State University of Paraíba, Campina Grande, Brazil (protocol No. 2.154.228), according to the guidelines contained in Resolution No. 466/12 of the National Health Council.

Study design and location

This documentary study was carried out through the analysis of the medical records of the victims of traffic accidents hospitalized in emergency wards in the municipality of Campina Grande, Paraíba, Brazil. The municipality has an estimated population of 407,472 inhabitants (data for 2018), a Human Development Index (HDI) of 0.72 and a Gini coefficient of 0.58.[12] The study was developed at the Dom Luiz Gonzaga Fernandes Emergency and Trauma Hospital in Campina Grande, Brazil – a referral institution for the care of trauma patients.

Inclusion and exclusion criteria

The inclusion criteria comprised the medical records of children of both sexes aged 0–12 years who were the victims of traffic accidents involving pedestrians, cyclists, motorcycles, and automobiles, and were hospitalized for at least 24 h. The medical records of the victims who were hospitalized at the time of data collection, and therefore were not yet available in the Medical Archive and Statistics Service, were excluded. Those that showed a percentage of missing information above 10% were also excluded.

Pilot study

A pilot study was conducted to evaluate the research methodology, to verify the reliability of the data collection instrument and to assess the presence of possible inconsistencies. The pilot study was carried out with 100 medical records of the victims of traffic accidents that happened in 2015; they were not used in the final sample.

Data collection

In total, 1,884 medical records of the traffic accident victims hospitalized in the period from January

to December 2016 were evaluated. Data collection was carried out by 3 properly trained researchers between May and December 2017. The following information was recorded: sex; age (0–4 years, 5–8 years, or 9–12 years); care shift (morning (06:00–11:59), afternoon (12:00–17:59), evening (18:00–23:59), or night (00:00–05:59)); day of the week; type of accident (motorcycle, automobile, cyclist, or pedestrian); presence of craniofacial trauma; trauma in other anatomical regions (ribcage, abdominal region, upper limbs, lower limbs, or hips); surgical treatment; admission to the intensive care unit (ICU); and outcome (discharge, transfer, or death).

Table 1. Distribution of the children according to sociodemographic characteristics, accident features and clinical characteristics

Variables	*n* (%)
Sex	
male	55 (64.7)
female	30 (35.3)
Age [years]	
0–4	16 (18.8)
5–8	32 (37.6)
9–12	37 (43.5)
Care shift	
morning (06:00–11:59)	12 (14.1)
afternoon (12:00–17:59)	27 (31.8)
evening (18:00–23:59)	41 (48.2)
night (00:00–05:59)	5 (5.9)
Day of the week	
Sunday	18 (21.2)
Monday	14 (16.5)
Tuesday	12 (14.1)
Wednesday	7 (8.2)
Thursday	15 (17.6)
Friday	9 (10.6)
Saturday	10 (11.8)
Type of accident	
motorcycle	32 (37.6)
automobile	13 (15.3)
cyclist	21 (24.7)
pedestrian	19 (22.4)
Craniofacial trauma	
yes	38 (44.7)
no	47 (55.3)
Other regions injured (*N* = 60)	
ribcage	3 (5.0)
abdominal region	6 (10.0)
upper limb	15 (25.0)
lower limb	32 (53.3)
hip	4 (6.7)
Surgical treatment	
yes	46 (54.1)
no	39 (45.9)
Admission to ICU	
yes	17 (20.0)
no	68 (80.0)
Outcome (*N* = 82)	
discharge	77 (93.9)
transfer	3 (3.7)
death	2 (2.4)

Unless marked otherwise, *N* = 85. ICU – intensive care unit.

Statistical analysis

The data was tabulated and analyzed using the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA). Data analysis involved descriptive (absolute and percentage distributions, mean (*M*), median (*Me*), and standard deviation (*SD*)) and inferential statistics (Pearson's χ^2 test). A significance level of 5% was adopted.

Results

The prevalence of traffic accidents affecting children was 4.5% (n = 85), with a mean age of 7.55 ±3.64 years and a median age of 8 years. The victims were predominantly males (64.7%), with a sex ratio of 1.8:1. Regarding age, the most frequently affected group was 9–12-year-olds (43.5%). Regarding the time of injury according to the care shift and the day of the week, most accidents were recorded in the evening (48.2%) and on the weekend (32.9%). Among all the victims, 37.6% were involved in motorcycle accidents. Craniofacial trauma occurred in 44.7% of the victims. Considering other anatomical regions, the highest frequency was observed for lower limb injuries (53.3%). Surgical treatment was required in 54.1% of cases. Only 20.0% of the victims required admission to ICU and 2.4% of the injuries resulted in death (Table 1).

Table 2 presents the distribution of craniofacial trauma injuries according to such variables as sex, age and type of traffic accident. Craniofacial trauma was significantly associated with motorcycle (p = 0.043) and automobile accidents (p = 0.013).

Table 2. Distribution of the child victims of traffic accidents involving craniofacial trauma according to sex, age group and type of accident

Variables	Craniofacial trauma		*p*-value
	yes (*N* = 38)	no (*N* = 47)	
Sex			
male	24/55 (43.6)	31/55 (56.4)	0.788
female	14/30 (46.7)	16/30 (53.3)	
Age [years]			
0–4	10/16 (62.5)	6/16 (37.5)	0.252
5–8	12/32 (37.5)	20/32 (62.5)	
9–12	16/37 (43.2)	21/37 (56.8)	
Motorcycle accident			
yes	10/32 (31.3)	22/32 (68.8)	0.043*
no	28/53 (52.8)	25/53 (47.2)	
Automobile accident			
yes	10/13 (76.9)	3/13 (23.1)	0.013*
no	28/72 (38.9)	44/72 (61.1)	
Cyclist accident			
yes	6/21 (28.6)	15/21 (71.4)	0.087
no	32/64 (50.0)	32/64 (50.0)	
Pedestrian accident			
yes	12/19 (63.2)	7/19 (36.8)	0.074
no	26/66 (39.4)	40/66 (60.6)	

Data presented as number (percentage) (*n*/*N* (%)). * statistically significant.

Discussion

Injuries resulting from traffic accidents are the 8th leading cause of death in all age groups, and the main etiology among children and young people from 5 to 29 years of age.[13] Epidemiological data indicates that low- and middle-income countries suffer from a higher burden of traffic injuries and deaths.[13] A report published as the global status report on road safety by the World Health Organization (WHO) revealed that systematic data collection in trauma databases, which gather information on the epidemiology of injuries, clinical interventions and health outcomes, is essential for preparing programs to improve clinical quality and for the development of effective prevention strategies.[13]

The present study, conducted in a referral center for the care of trauma patients, revealed a low percentage (4.5%) of child victims of traffic accidents. According to the Mortality Information System of the Brazilian Ministry of Health, in the years 2014–2018, 3.5% of the total number of deaths from traffic accidents (192,686) regarded children aged up to 14 years, with a decline of almost 40.0% in the period.[14] This result demonstrates the importance of measures that were implemented in the country to control this type of accidents after the adoption of the Brazilian Traffic Code in 1997, which defined attributions to the agencies linked to traffic, and established norms and penalties for road users.[15] The use of car seats for infants and young children, and mandatory rear seat transportation for children up to 10 years of age[16] may have contributed to the lower frequency of accidents in this population group.

The majority of victims in this study were males, which is consistent with data from WHO,[13] and with previous studies carried out by researchers in Brazil,[10,17–19] Sudan,[6] Portugal,[20] and India.[21] It could be inferred that this male predominance exists because of cultural and social restrictions for unsupervised female outdoor activities as compared to male activities.[6,22]

The incidence of transport accidents was higher among older children. While younger children generally suffer from trauma due to low-intensity forces, such as falls, older children are more likely to be exposed to high-intensity forces, such as those resulting from traffic accidents, because as they grow, their trauma profile becomes similar to that observed in adults.[4,7] In addition, when they are young, children are protected by their parents against harmful events.[3]

Regarding the time and day of the week of traffic accidents, the concentration of events in the evening and on weekends corroborates other findings.[10,17,23,24] The greater number of accidents at dusk may be associated with poor visibility, stationary vehicles, speeding, disrespect for signs, and the use of alcohol or drugs.[23] The higher occurrence of accidents on weekends may be related to risky traffic behavior, such as exceeding the speed limit, disrespecting traffic rules and driving under the influence of alcohol.[10] However, the Brazilian "dry law" from 2008 reduced the legal alcohol blood level from 0.06 g/dL to 0.02 g/dL.[16,25] Later, in 2012, any alcohol blood concentration among drivers was considered illegal.[26] These measures proved to be important in reducing hospital admissions due to traffic accidents, the length of hospital stay and hospital expenses.[27]

Regarding the type of vehicle involved in the accident, motorcycles were the most common, as reported by other authors,[10,18,28] followed by accidents involving cyclists and pedestrians. According to WHO, more than half of all traffic deaths involve vulnerable road users, such as pedestrians, cyclists and motorcyclists.[13] In the present study, the prevailing involvement of motorcycles in traffic accidents might be due to the fact that this vehicle is the predominant means of transportation for the population of a lower socioeconomic status in small- and medium-sized Brazilian cities.[10] Other factors, such as little or no protection used by motorcyclists,[17] difficulty in making motorcycles visible for other drivers, inappropriate traffic behavior, and the lack of respect for traffic laws,[28] may contribute to the higher number of traffic accidents involving motorcycles.

Thus, to reduce the number of traffic accidents, additional informational and educational measures must be taken, and safer protection devices must be developed.[29] In fact, correct helmet use can reduce the risk of fatal injuries by up to 42% and the risk of head injuries by 69%.[13] Fastening seat belts reduces the mortality of front seat occupants by 45–50%, and the risk of death and serious injury among rear seat occupants by 25%. In addition, restrictive laws for children regarding their seating in vehicles can lead to a 60% reduction in deaths.[13] The prevalence of craniofacial trauma was similar to that reported in a previous study.[18] Factors such as speed, the victim's position, the lack of use of safety devices, and impact geometry are mainly responsible for injuries due to traffic accidents.[30]

Statistically significant associations between craniofacial trauma and accidents involving motorcycles and automobiles were observed. Motorcyclists are vulnerable to these injuries due to little or no protection during an accident.[17] In addition, in general, automobile accidents produce greater impact than other types of accidents, resulting in more serious and complex craniofacial injuries.[3]

Limitations

There are some limitations to this study. It was impossible to investigate cause-and-effect relationships due to the cross-sectional design, and there may have been incomplete data from some of the medical records. Nevertheless, this research presents relevant epidemiological data about traffic accidents as a public health problem in children up to 12 years of age, providing knowledge about the risk factors for craniofacial trauma, so that better surveillance and continuous education on traffic safety can be implemented.

Conclusions

Older male children are the predominant victims of traffic accidents. There is a greater frequency of accidents involving motorcycles, with a high prevalence of craniofacial trauma.

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Are there any relationships between personality type, salivary total antioxidant level and academic stress?

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Abstract

Background. Stress is one of the most important determinants of total antioxidant capacity (TAC).

Objectives. This study aimed to assess alterations in salivary TAC following academic stress according to the personality type.

Material and methods. This descriptive study evaluated 53 dental students at Shahid Beheshti University of Medical Sciences, Tehran, Iran, who were divided into type A ($n = 25$) and type B ($n = 28$) personality groups using the Bortner questionnaire. Saliva samples were collected during the 1st week of the semester (a low-stress period) and during the 1st week of the final exams (a high-stress period). Salivary TAC was measured using a specific kit. The data was analyzed using the repeated measures analysis of variance (ANOVA), the analysis of covariance (ANCOVA), the χ^2 tests, the independent t tests, and the Bonferroni adjustments.

Results. Overall, salivary TAC in the high-stress period was significantly lower than that in the low-stress period (0.27 vs 0.31 mM) ($p = 0.016$). The comparison of salivary TAC between the type A and type B personality groups in the low-stress period showed no significant difference ($p = 0.450$). During the high-stress period, a reduction in salivary TAC was recorded in both groups, which was borderline significant in the type A personality group ($p = 0.050$), but non-significant in the type B personality group ($p = 0.140$). The comparison of salivary TAC between the type A and B personality groups in the high-stress period also revealed no significant difference ($p = 0.780$).

Conclusions. Academic stress can decrease salivary TAC, and the personality type has no significant effect on this relationship.

Keywords: stress, saliva, antioxidant, personality type

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Introduction

Oxidative stress is due to the impaired balance between the production of free radicals and reactive oxygen species (ROS) and antioxidant defense mechanisms. This imbalance can adversely affect various biomolecules, including lipids, proteins and nucleic acids. Oxidative stress can result from the overproduction of free radicals and ROS, or a decreased production of antioxidants.[1–5] Oxidative stress can ultimately damage cells and organs, or impair their function.[3] From a biomedical point of view, an antioxidant is a material that, when present at a much lower concentration as compared with that of an oxididant, can significantly prevent or delay the oxidation reaction.[6]

Considering the fact that the antioxidant capacity of particular antioxidants depends on the individual capability of these enzymes to counteract oxidative stress, it seems that the evaluation of each antioxidant alone would not yield conclusive results. Moreover, there are many types of antioxidants, and their individual assessment would be difficult and costly. Thus, total antioxidant capacity (TAC) is commonly measured in current research studies.[6] On the other hand, the collection of saliva and urine samples is non-invasive and easier as compared to blood collection. Therefore, in recent decades, saliva and urine samples have been more commonly used than blood samples for the assessment of TAC.[2,5] Moreover, it has been confirmed that a decreased salivary TAC indicates oxidative stress. In other words, the level of oxidative stress can be determined by measuring salivary TAC.[7]

A reduction in TAC can negatively affect general health.[7] This reduction can be influenced by both physical and psychological parameters. Considering a psychological aspect, it seems that psychological stress, anxiety, depression, poor coping mechanisms, and many other psychological variables are directly related to oxidative stress, and can subsequently affect TAC.[8,9] Among various stressors, academic stress refers to a type of psychological stress that can cause a predictable sense of hopelessness and academic failure, even unconsciously.[10] It can be evoked by many factors associated with the academic environment. For example, academic stress in medical students can be due to exams, falling behind in studying, a lack of time to review the course material, heavy workload, the need to actively participate in classes, and requirements for good academic performance.[11] Increased oxidative stress may increase psychological fatigue, and subsequently decrease learning ability.[12] Ito et al. found an inverse correlation between the blood levels of reactive oxygen metabolites 3 days before national examinations and the final scores of students.[12]

The personality of each individual is made up of a mixture of specific characteristics.[13] Considering variability in personality traits and the fact that the personality of each individual is an important predictor of their health status, it seems that stress is also related to the characteristics forming personality. Thus, in most cases, the personality type is a good predictor of the stress status. However, reports in this respect have been contradictory.[11,13] In 2014, Bob et al. reported that an anxious and nervous personality was significantly positively correlated with the level of academic stress.[11] According to Batti et al., as the unique patterns of feeling, thinking and behaving shape the personality of an individual, personality traits determine to a great extent the university student's ability to handle academic issues as well as their general performance.[14] For example, Sakitri showed that type A personality had a positive effect on academic performance.[15]

The original version of the Bortner personality type questionnaire was developed in 1969.[16] It contains 14 items, each consisting of 2 phrases placed at the opposite ends of a continuum ranging from an extreme type A behavior pattern (TABP) to the absence of TABP.[16] Type A personality is characterized by ambition, aggressiveness, impatience, competitiveness, anxiety, and restlessness. Time is valuable to such people, and thus they are nervous and hasty. These individuals are also fearless, often intolerant, and can be workaholics due to their high energy levels. Type A individuals resist accepting their situation, and often suffer when they cannot control the situation or people around them. In contrast, individuals with type B personality are calm and present lower levels of stress. They are cool, flexible and emotional.[13,17,18] According to a study by Catipović-Veselica, the levels of aggressiveness and suspicion in individuals with type A personality are higher than in the case of type B personality; however, these groups are not significantly different regarding sociability and depression.[19]

People living in different communities have different psychological conditions. Considering the limited number of studies on the effect of academic stress on salivary TAC and the lack of information regarding the possible influence of the personality type on alterations in salivary TAC, this study aimed to assess changes in the salivary TAC of dental students following academic stress associated with final examinations, and the role of the personality type in this respect.

Material and methods

This descriptive, cross-sectional study evaluated 53 dental students attending Shahid Beheshti University of Medical Sciences, Tehran, Iran. According to a previous study by Pani et al.,[7] the minimum sample size required to detect significant differences between the groups was calculated to be 24. The alpha error and the study power were 0.05 and 80%, respectively. The study protocol was approved by the ethics committee at Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.REC.1397.025). Out of the 53 students included in the study, 25 had type A and 28 had type B personality.

The 2 groups were matched in terms of gender and age. The study population included 3rd–6th year dental students, of which 92.44% were 4th or 5th year dental students. The inclusion criteria for the study were as follows:

– non-alcoholics;
– the absence of malnutrition (according to the World Health Organization (WHO), malnutrition refers to deficiencies, excesses or imbalances in an individual's energy and/or nutrient intake; considering the body mass index (BMI) formula (weight in kilograms/height in meters squared), individuals with BMI < 18.5 have malnutrition; such individuals were excluded from the study);
– no pregnancy;
– good general health and the absence of systemic diseases that are known to affect salivary TAC;
– the absence of oral mucosa lesions at the time of sampling; and
– willingness to participate in the study.

Therefore, individuals with a history of alcohol consumption, malnutrition, pregnancy, systemic diseases, the presence of oral mucosa lesions at the time of sampling, and disinclination to participate were excluded from the study.

According to various studies, the 1st week of the semester is considered the period of the lowest academic stress, while the 1st week of final exams is considered the period of the highest academic stress.[7,20,21] In the 1st week of the 1st semester of the academic year 2018/2019, the questions concerning demographic data and the Bortner questionnaire (self-assessment of the personality type) were administered to 3rd–6th year dental students. A total of 60 students participated in the initial phase of the study, of which 30 had type A and 30 had type B personality. Also, saliva samples were collected from the participants at this time, and then again during the 1st week of final exams. Overall, 25 students with type A and 28 students with type B personality showed up for the 2nd round of saliva sampling. The remaining 7 participants were excluded from the study due to poor cooperation.

Saliva collection

Unstimulated saliva samples were collected as described by Navazesh and Kumar.[22] Saliva samples were collected in a resting seated position between 9 a.m. and 12 p.m. The participants were requested to refrain from eating, drinking and cigarette smoking for 2 h prior to saliva collection. They were requested to rinse their mouth with tap water, wait for 2 min for saliva to collect in their mouth, and then spit repeatedly into a test tube until 5 mL of saliva was collected. The collected samples were then immediately placed on ice and sent to a laboratory. The samples were centrifuged at 4°C for 10 min to remove debris, and then the saliva was frozen at –80°C for later testing.[22,23]

Measurement of salivary TAC

Salivary TAC was measured using a TAC assay kit (96 test; ZellBio, Lonsee, Germany). This kit measures TAC via a comparison with the activity of ascorbic acid as the standard. Its mechanism of action is based on redox colorimetry in a diagnostic range of 0.125–2 mM (M = mol/L). This kit can measure TAC with an accuracy of 0.1 mM.[23]

Bortner questionnaire

The personality type of dental students was determined using the Bortner questionnaire. This questionnaire evaluates type A and type B personality. The Bortner ordinal scale includes 14 items. Each item is composed of 2 clauses that are at the 2 ends of an 11-point Likert scale. The score obtained by each individual may range from 0 to 140. A score of 70 was considered as the cut-off point to separate type A and type B personality, with scores 0–70 being closer to type B rather than type A personality. Individuals with lower scores have a stronger type B personality. In addition, the translated Persian version of this questionnaire has been used in many studies, and its reliability and validity have been confirmed in Iranian subjects.[24]

In order to have an acceptable sample size, we did not consider smoking as an exclusion criterion. Nonetheless, the possible confounding effect of this variable was controlled in the statistical analysis of the data.

Statistical analysis

The data was analyzed using the IBM SPSS Statistics for Windows software, v. 21.0 (IBM Corp., Armonk, USA). The variables were compared using the repeated measures analysis of variance (ANOVA), the analysis of covariance (ANCOVA), the χ^2 tests, the independent t tests, and the Bonferroni adjustments.

Results

The 2 groups with type A and type B personality were matched in terms of gender (p = 0.770). In the type A personality group, 16 (64.0%) were females and 9 (36.0%) were males, while in the type B personality group, 19 (67.9%) were females and 9 (32.1%) were males. The mean age of the participants was 22.91 ±1.33 years, and the 2 groups were also matched in this respect (p = 0.590). The mean age of the participants in the type A and type B personality groups was 22.80 ±1.15 years and 23.00 ±1.49 years, respectively.

There were 7 smokers in this study (4 in the type A personality group and 3 in the type B personality group). ANCOVA showed that their presence in the study had no confounding effect on the results (p = 0.910).

In general, salivary TAC was decreased during the high-academic stress period as compared to the low-academic stress period (0.27 ±0.10 mM vs 0.31 ±0.09 mM; a reduction of 0.04 mM), irrespective of the personality type, and this reduction was statistically significant (p = 0.016).

The comparison of salivary TAC within the type A personality group between the low- and high-academic stress periods revealed that salivary TAC in the high-academic stress period decreased as compared to the low-academic stress period, and this reduction was borderline significant (p = 0.050) (Table 1).

Table 1. Salivary total antioxidant capacity (TAC) in the participants during the low- and high-academic stress periods (comparisons within the groups)

Group	Salivary TAC [mM]	*p*-value
A_1	0.32 ±0.08	0.050*
A_2	0.27 ±0.10	
B_1	0.30 ±0.11	0.140
B_2	0.26 ±0.11	

Data presented as mean ± standard deviation (*M ±SD*);
A_1 – type A personality group during the low-academic stress period;
A_2 – type A personality group during the high-academic stress period;
B_1 – type B personality group during the low-academic stress period;
B_2 – type B personality group during the high-academic stress period;
* borderline significant.

Salivary TAC was not significantly different in the type A and type B personality groups during the low-academic stress period, although it was slightly lower in the type B group (p = 0.450). Similarly, salivary TAC was not significantly different in the type A and type B personality groups during the high-academic stress period, although it was slightly lower in the type B group (p = 0.780) (Table 2).

Table 2. Salivary total antioxidant capacity (TAC) in the participants during the low- and high-academic stress periods (comparisons between the groups)

Group	Salivary TAC [mM]	*p*-value
A_1	0.32 ±0.08	0.450
B_1	0.30 ±0.11	
A_2	0.27 ±0.10	0.780
B_2	0.26 ±0.11	

Data presented as *M ±SD*.

The comparison of salivary TAC within the type B personality group between the low- and high- academic stress periods revealed that salivary TAC in the high-academic stress period decreased as compared to the low-academic stress period, but this reduction was non-significant (p = 0.140) (Table 1).

ANCOVA also revealed that the salivary TAC of the participants was not affected by their personality type (p = 0.980).

Discussion

This study assessed changes in salivary TAC during low- and high-academic stress periods based on the personality type of dental students. The mean age of the participants was 22.91 ±1.33 years. In this study, irrespective of the personality type, salivary TAC decreased in the high-academic stress period as compared to the low-academic stress period, indicating that academic stress due to exams affects salivary TAC, although it only lasts for a short period of time (the exam season). This finding is in agreement with the results of Pani et al.; these authors also showed that salivary TAC significantly decreased during academic exams.[7] It should be noted that different academic fields may cause different stressful conditions. Akbari et al. reported that 52% of dental students had abnormal levels of stress during the course of their education.[25] It was also shown that academic stress had a significantly greater effect on the personal stress levels of dental students as compared to other factors.[25] In contrast, Ito et al. found no significant change in the levels of oxidative stress derivatives in the blood samples taken from students 3 weeks and 3 days before exams.[12] However, it should be noted that this latter study assessed blood TAC, and not salivary TAC.

In this study, salivary TAC in the type A personality group decreased in the high-academic stress period as compared to the low-academic stress period, and this reduction was borderline significant. A similar reduction was noted in the type B personality group, but it did not reach statistical significance. Considering the characteristics of individuals with type A personality, such as higher levels of anxiety and stress than in the case of type B personality, it is possible that individuals with type A personality are more affected by academic stress due to exams. However, this effect did not seem to be highly remarkable. The reason for this may be the competitive spirit of these individuals, which may neutralize their stress. Similar results were also reported by Bob et al.[11] Further studies are required to better investigate this topic.

A search of the literature yielded no studies examining the correlation between the personality type and changes in TAC during academic stress periods to compare our results with. However, Moriana and Herruzo reported that individuals with type A personality experienced higher levels of anxiety.[26] Also, anxiety can suppress antioxidant defense mechanisms.[27] On the other hand, Wilkinson et al. found no significant correlation between the academic performance of students in exams and their flexibility, anxiety, stress, and depression.[28] Chai and Low also reported that psychological stress was not affected by the personality type.[29] In addition, Matos-Gomes et al. found that the concentration of total protein in participants was not significantly different between 2 groups – with and without psychological stress – during low- and high-academic stress periods.[20]

It seems that individuals may find ways to cope with life stressors in their personal life experiences. In other words, they acquire experience, expertise and skills to deal with a specific stressor. Such variations in the results across studies may be due to multiple reasons, e.g., different methodologies, different methods for the assessment of salivary TAC or individual antioxidants, the type of kits used for this purpose, the inclusion and exclusion criteria, the sample size, the use of stimulated or unstimulated saliva, the collection of urine, blood or saliva samples, geographical location, or the socioeconomic status of the participants.

According to the present results, salivary TAC during the high-academic stress period was not affected by the personality type, and only depended on salivary TAC in the low-academic stress period. In other words, individuals with a higher salivary TAC in the 1st week of the semester also showed a higher salivary TAC during the high-academic stress period. Since the salivary TAC of the individuals in the 2 groups was not significantly different in the low-academic stress period, the difference in the high-academic stress period was still non-significant.

One strategy to decrease the level of stress in students is to change the method of assessment of their academic performance. For instance, Ali et al. found that stress levels in students whose performance in exams was reported as pass/fail were lower in comparison with students whose performance in exams was reported as a grade point average.[30] This factor can also be considered as a potential confounder in previous studies.

Limitations

Finally, it is necessary to state that this study has several limitations. Firstly, only one aspect and one tool assessing the specific traits of personality were used. We did not assess the study groups in terms of personality disorders, which also could be an important confounder. In addition, using only self-assessment tools to determine personality traits may introduce bias. Therefore, it is recommended that future studies use clinical examinations, such as properly structured interviews, to assess personality in a more comprehensive way.

Conclusions

This study showed that academic stress could significantly decrease salivary TAC, and that personality type had no significant effect on this relationship.

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Stress distribution around dental implants, generated by six different ceramic materials for unitary restoration: An experimental photoelastic study

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Abstract

Background. Various ceramic materials have been used for esthetic rehabilitation with implants, but the issues regarding the dissipation of masticatory loads are not well understood.

Objectives. This in vitro quasi-static study aimed to evaluate with the photoelasticity test the dissipation of stress around dental implants with regard to different rehabilitation materials.

Material and methods. A photoelastic model was elaborated in resin, where a conical Morse-tapered implant was inserted. On the abutments (1 per crown), 6 single crowns were prepared using different materials to form 6 groups: feldspathic ceramic (G1); chrome-cobalt alloy covered with ceramic (G2); hybrid ceramic (G3); zirconia covered with ceramic (G4); zirconia (G5); and lithium disilicate (G6). Axial loads of 100 N (load 1) and 300 N (load 2) were applied in the center of the crowns, and photoelastic images were captured and analyzed. The total area of stress dissipation was measured for each group. Then, a computational program was developed to measure the number of pixels of the colors generated in each group. Two image sizes were analyzed – total image and crestal image.

Results. Counting the numbers of pixels of the colors in the total images showed that G6 > G4 > G5 > G1 > G2 > G3 when load 1 was applied. When load 2 was applied, the sequence was G6 > G4 > G1 > G3 > G2 > G5. In the evaluation of the crestal area, the obtained results were G4 > G5 > G1 > G3 > G2 > G6 with load 1 and G5 > G1 > G2 > G6 > G4 > G3 with load 2.

Conclusions. Within the limitations of this in vitro quasi-static study, the findings indicate that the zirconia crown (G5) presented higher stress in the crestal images, while the lithium disilicate crown (G6) presented higher stress in the total images.

Keywords: dental implant, ceramic materials, computer-aided design/computer-aided manufacturing, photoelastic stress analysis, stress dissipation

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Introduction

The ongoing search for the ideal restorative material in terms of resistance characteristics, esthetics and biocompatibility has led to the development of new materials in modern restorative dentistry. The innovations have been implemented in implantology as well. However, at present, we can affirm that there is no consensus on the ideal material that adapts to all cases. The most esthetic materials have lower flexural strength and vice versa (Fig. 1).[1]

Describing more specifically some of the ceramic materials frequently used for esthetic restorations, feldspathic ceramic has excellent esthetic qualities, but it has lower resistance than other ceramic materials; this difference is more notable in implantology due to the impossibility of increasing resistance by performing adhesive cementation on the dental substrate. Since lithium disilicate presents acceptable levels of both resistance and esthetics, it is indicated for single implant restorations.[2,3] Zirconia is already widely accepted as a restorative material due to its biocompatibility and resistance properties, although it has poorer esthetic qualities as compared to the previously mentioned materials.[4,5]

Actually, there is controversy about the use of zirconia without ceramic coating due to its great hardness. There is a possibility of excessive wear of the opposing part and of the transmission of loads to the supporting structures in the case of materials with such hardness. Hardness, in addition to the surface roughness and tenacity of restorative materials, is one of the factors considered as determinants of the enamel wear caused by antagonistic teeth.[6] The Vickers hardness value for zirconia is 1,250 HV, which is well above the hardness of the enamel (275 HV) or the dentin (66 HV), and also of other frequently used materials, such as composite (87–124 HV), feldspathic porcelain (700 HV), lithium disilicate (590 HV), titanium (349 HV), and gold (130–135 HV).[7] In theory, using a material with a lower modulus of elasticity would transmit less stress to the supporting structures. Some authors recommend the use of more resilient materials to absorb part of the impact exerted on implants.[8,9] Other authors recommend the use of acrylic resin teeth for the full-arch prostheses restored over implants, since this type of material would compensate for the lack of resilience of this rehabilitation system, which is different from natural teeth, as natural teeth have periodontal ligaments.[10,11]

In a finite element analysis study evaluating stress distribution in the supporting structures with regard to materials of different hardness, the researchers showed that both resin types (composite and ceramic-filled resin) transmitted less load than porcelain or the gold alloy.[10] Other finite element analysis studies were unable to demonstrate benefits in stress distribution in the supporting structures with the use of more resilient materials.[12] On the contrary, some authors demonstrated that the most resilient materials increased stress in the prosthetic fastening screws.[13] Hence, until now, there has been no consensus on which material is ideal from a biomechanical point of view. Thus, the objective of this study was to evaluate by means of the photoelastic stress analysis, applying a quasi-static axial load, the dissipation of stress in the supporting structures of the implant restoration with a unitary crown. Six different materials with different elasticity modules were tested for unitary crowns: feldspathic ceramic; metal-ceramic; polymer-filled ceramic; ceramic-stratified zirconia; monolithic zirconia; and lithium disilicate. The hypothesis tested was that the material used for the manufacture of the crown does not affect the pattern of load dissipation in the peri-implant bone tissue.

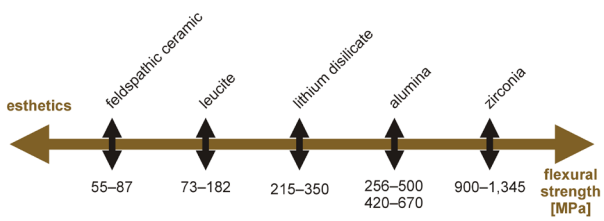

Fig. 1. Representative scheme showing that highly esthetic materials present lower flexural strength and vice versa

Material and methods

This in vitro study used 1 conical implant with a Morse-tapered connection, with dimensions of 11 mm in length and 4 mm in diameter, and 6 abutments (1 per crown) – 4.5 mm in diameter, 6 mm in height and 3.5 mm in the transmucosal length. All pieces were manufactured by Implacil De Bortoli (São Paulo, Brazil) and are demonstrated in Fig. 2.

Development of the experimental models

Initially, the implant was installed in a wooden block with dimensions of 12 mm in thickness, 25 mm in height and 30 mm in length at a depth of 2 mm from the surface of the block, according to the manufacturer's recommendations.

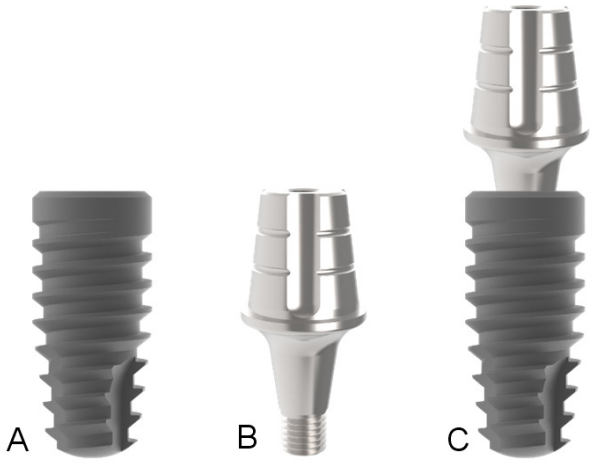

Fig. 2. Representative images of the pieces used in the study

A – conical implant; B – abutment; C – set with both pieces connected (implant and abutment).

Then, the abutment was screwed to the implant and a silicone impression was made with the use of the transfer abutment indicated for the system, generating a mold for the inclusion of the implant in resin. The implant–abutment set was positioned with the abutment connected to the transfer abutment inside the silicone mold and resin was poured, filling all spaces. A flexible epoxy resin model G4 (Polipox, São Paulo, Brazil) was used. After the complete polymerization of the resin, the block was polished using a sequence of sandpaper and resin polishing pastes. Figure 3 shows the resin block with the implant–abutment set.

Crown preparation and group formation

A plastic cap corresponding to the abutment dimensions was installed over 1 abutment, and a dental crown corresponding to a lower right first molar, with dimensions equal to the natural anatomical measurements described for this dental element (11.4 mm in the mesiodistal direction, 10.2 mm in the buccal-lingual direction and 7.7 mm in height),[14] was waxed and polished. Then, a matrix (barrier) was made with silicone for the elaboration of the crowns in which ceramic was applied manually (the metal-ceramic and zirconia-ceramic crowns). In addition, the crown and the abutment were scanned using a dental scanner (Cerec® AC; Dentsply Sirona, Behnheim, Germany) for the subsequent milling of the crowns in 5 different materials and the preparation of the metal-ceramic crown, which was fabricated using the conventional method. Six groups were formed according to the material used: feldspathic ceramic (G1); metal-ceramic (VITA VM9; VITA Zahnfabrik, Bad Säckingen, Germany) (G2); hybrid ceramic (Shenzhen Upcera Dental Technology Co. Ltd., Shenzhen, China) (G3); yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) covered with ceramic (VITA VM9; VITA Zahnfabrik) (G4); partially stabilized Y-TZP (inCoris TZI; Dentsply Sirona) (G5); and lithium disilicate (Shenzhen Upcera Dental Technology Co. Ltd.) (G6). Figure 4 shows the crowns fabricated for each group, positioned on the abutments.

Fig. 3. Block with the implant–abutment set finished for the tests

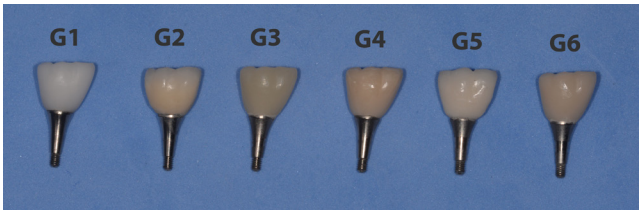

Fig. 4. Crowns fabricated for each group, positioned on the abutments
G1 – feldspathic ceramic; G2 – metal covered with ceramic; G3 – hybrid ceramic; G4 – zirconia covered with ceramic; G5 – zirconia; G6 – lithium disilicate.

Photoelastic test, image acquisition and data collection

To test the sample of each group, an abutment was installed on the implant and torqued at 25 N. Then, the crown was cemented using resin cement (RelyX™ Ultimate; 3M ESPE, St. Paul, USA) and subjected to a load of 5 kgf for 5 min. Therefore, a new abutment was used for each tested group, as it would be difficult to remove the crown cemented over the abutment. For the photoelasticity test, each sample was placed on a polariscope with circular crossed polarizers (Meadowlark Optics, Inc., Frederick, USA) and 2 occlusal axial load intensities were applied – 100 N (load 1), and 300 N (load 2). The loads were applied in the central pit of each crown, using a hydraulic press coupled with a load cell. Each load was measured with a calibrated load cell (Model 2000; OHAUS Corporation, Pine Brook, USA). Images were obtained for each group, for both applied loads, using a Nikon camera, model D3200 (Tokyo, Japan), which was fixed in the same position (distance and angulation) in relation to the block.

First, the total dissipation area (discounting the implant area, which is equal to 40 mm^2) of the applied forces was measured for each load, as shown in Fig. 5. The area in each image was measured twice by each author, generating a total of 8 measurements for each image. These measurements were performed using the ImageJ software (National Institutes of Health, Bethesda, USA).

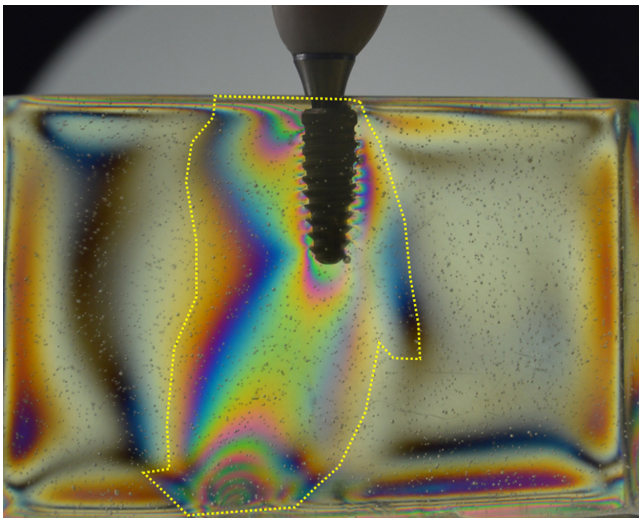

Fig. 5. Representative image of the measured area of stress dissipation around the implant

Then, to quantify the fringes in the total images, all images were standardized to 12 mm in width and 16 mm in height, as shown schematically in Fig. 6. For the crestal area, the images were standardized to assess cervical loads as follows – from the implant platform to the 3rd thread (4 mm) and at a distance of 4 mm from the implant platform – as shown schematically in Fig. 7. A program was developed for the evaluation and quantification of the main colors of the fringes in each image.

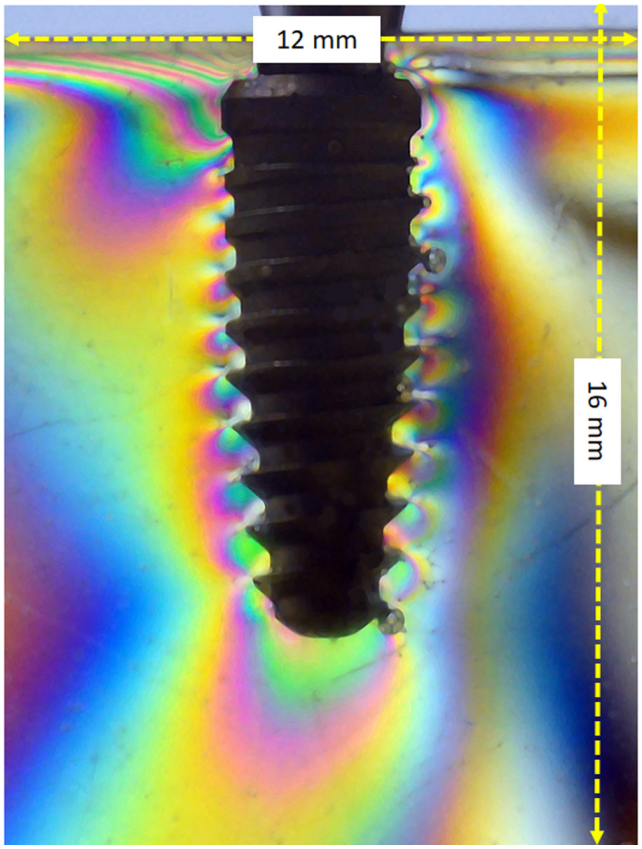

Fig. 6. Representative image of the total area standardized to measure the color quantity with the use of the computational program

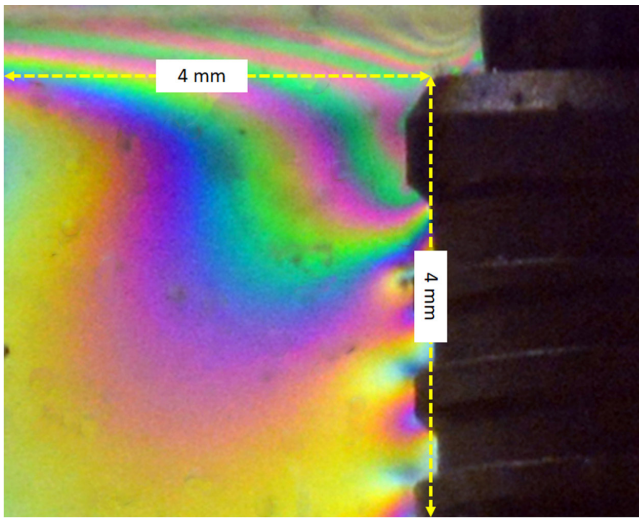

Fig. 7. Representative image of the crestal area standardized to measure the color quantity with the use of the computational program

Development of a computational program

In order to analyze the distribution of fringes in the images, a Python application was created using the scikit-image processing library.[15] The application takes an image, a color and 2 parameters as the input. The color parameters are given as the a* and b* values of a color in the International Commission on Illumination (Commission internationale de l'éclairage – CIE) L*a*b* color space[16]; the 2 remaining parameters are the lightness parameter λ and the error parameter ε. The input image can be given in the RGB (red, green and blue) color space, but it is then converted to the CIE L*a*b* color space, using the standard illuminant, so that the color variables of each pixel can be analyzed independently of the varying luminosity.

A new monochromatic image of the same proportions as the original one is created and initially consists entirely of black pixels. The application takes each individual pixel, represented by its CIE L*a*b* color space coordinates (L*, a* and b*), of the converted image and checks it against the following conditions (Inequality 1 and Inequality 2):

$$L < \lambda \tag{1}$$

$$\sqrt{(a - a_0)^2 + (b - b_0)^2} < \varepsilon \tag{2}$$

where:
L – lightness coordinate of the pixel;
a – a* coordinate of the pixel;
b – b* coordinate of the pixel;
a_0, b_0 – a* and b* values of the input color in the CIE L*a*b* color space;
λ – lightness parameter;
ε – error parameter.

For each pixel of the input image that satisfies the above conditions, the corresponding pixel in the generated monochromatic image becomes white. In this manner, by setting the input colors to a_0 = −128;0;0 and b_0 = 0;−128;128 for green, blue and yellow, respectively, and setting the lightness and error parameters according to each image, we isolated the green, blue and yellow fringes of each image. An example can be seen in Fig. 8.

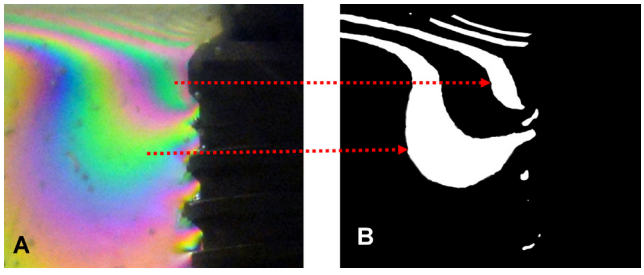

Fig. 8. Illustrative image showing the isolated green fringes generated by the Python application

A – distribution of all fringes during load application; B – isolation of green fringes for the measurement.

Next, the white area of each monochromatic image was measured by simply counting the number of white pixels and dividing it by the total number of pixels.

Statistical analysis

The data was compared statistically using the one-way analysis of variance (ANOVA) to verify differences between the 6 groups with regard to the 2 proposed conditions (load 1 and load 2). Pearson's correlation test was applied to check for the correlation between the measured area of stress dissipation and the number of pixels in each group. A p-value <0.05 was considered statistically significant. All data was analyzed using the GraphPad Prism software, v. 5.01 for Windows (GraphPad Software Inc., San Diego, USA).

Results

Load distribution in the resin base, which simulates the bone tissue, varied in the 6 groups, showing statistically significant differences between the values with regard to the tested load conditions ($p < 0.0001$). Under load 1, G3 showed the lowest stress dissipation around the implant, while under load 2, G5 showed the lowest stress dissipation around the implant. The values of the area of stress dissipation for each group are shown in Table 1. Figure 9 graphically shows the data for all groups with regard to the 2 loads.

The variation in the number of pixels measured for the colors was similar to the variation in the area of strain distribution measured for each group. According to the values of strain distribution, measured by counting the number of pixels of the colors in the total images, the sequence of the groups was G6 > G4 > G5 > G1 > G2 > G3 when load 1 was applied, showing a difference of 75.5% between the highest and the lowest values. When load 2 was applied, the sequence was G6 > G4 > G1 > G3 > G2 > G5, showing a 19.8% difference between the highest and the lowest values. In the evaluation of the crestal area, the obtained results were G4 > G5 > G1 > G3 > G2 > G6 when load 1 was applied, showing a 108.9% difference between the highest and the lowest values, and G5 > G1 > G2 > G6 > G4 > G3 when load 2 was applied, showing a difference of 5.6% between the highest and the lowest values. The values for each group are shown in Table 2.

Applying Pearson's correlation test, a positive correlation was detected between the data for the total area and the total number of pixels (Fig. 10).

In assessing the number of pixels corresponding to each evaluated color tone, the yellow tone was the one that varied the most between the 2 load intensities in all groups and in both image sizes. Figure 11 shows the distribution of the total number of pixels measured for each color in both image sizes.

Table 1. Area of stress dissipation [mm^2] in each group for the 2 loads

Group	Load 1	Load 2
G1	27.6 ±0.39	307.2 ±0.27
G2	26.6 ±0.36	301.9 ±0.33
G3	20.1 ±0.66	306.4 ±0.42
G4	31.5 ±0.34	309.2 ±0.21
G5	26.1 ±0.66	287.3 ±0.34
G6	34.5 ±0.34	317.3 ±0.20

Data presented as mean ± standard deviation (*M* ±*SD*). Load 1 – 100 N; load 2 – 300 N.

Table 2. Number of pixels in each group for the 2 loads, in both image sizes (values considering all color tones)

Group	Total image		Crestal image	
	load 1	load 2	load 1	load 2
G1	275,794	750,568	179,964	543,929
G2	264,908	702,581	144,847	537,600
G3	203,802	740,019	168,309	521,267
G4	328,731	766,634	231,316	531,460
G5	277,551	689,950	216,862	550,291
G6	357,697	826,453	110,684	535,137

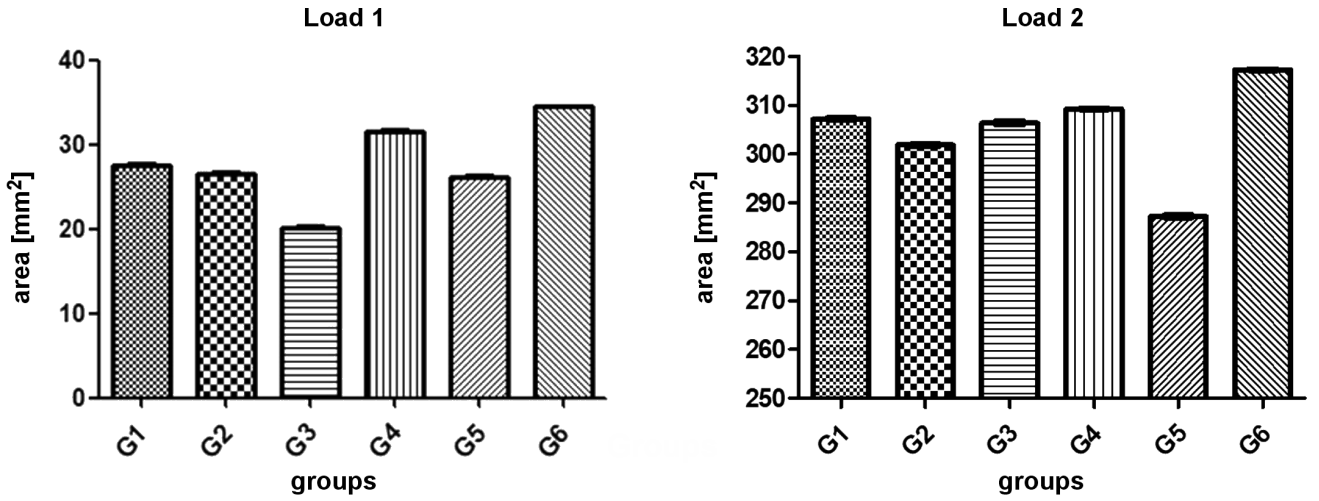

Fig. 9. Bar graphs showing the area of stress dissipation for all groups with regard to the 2 loads

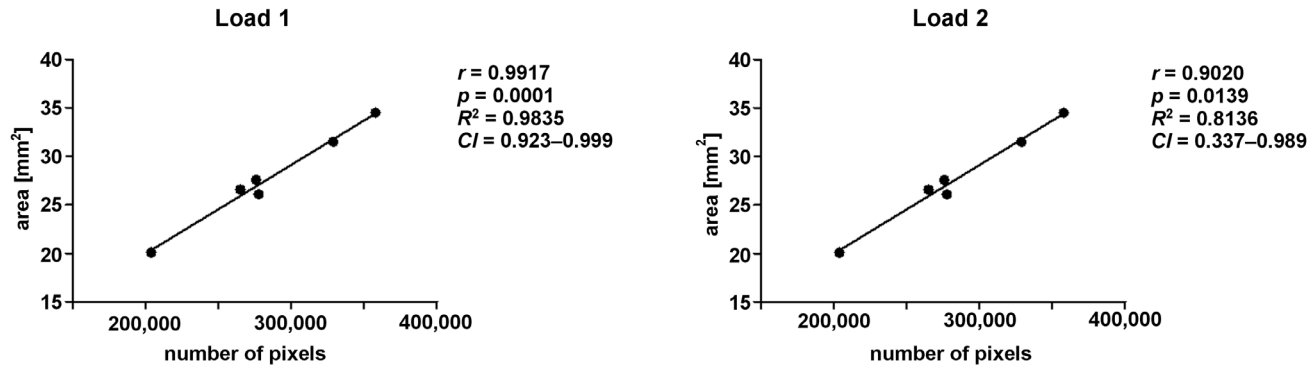

Fig. 10. Correlation between the data for the total area and the total number of pixels for the 2 loads (Pearson's correlation test)
r – Pearson's correlation coefficient; R^2 – coefficient of determination; *CI* – confidence interval.

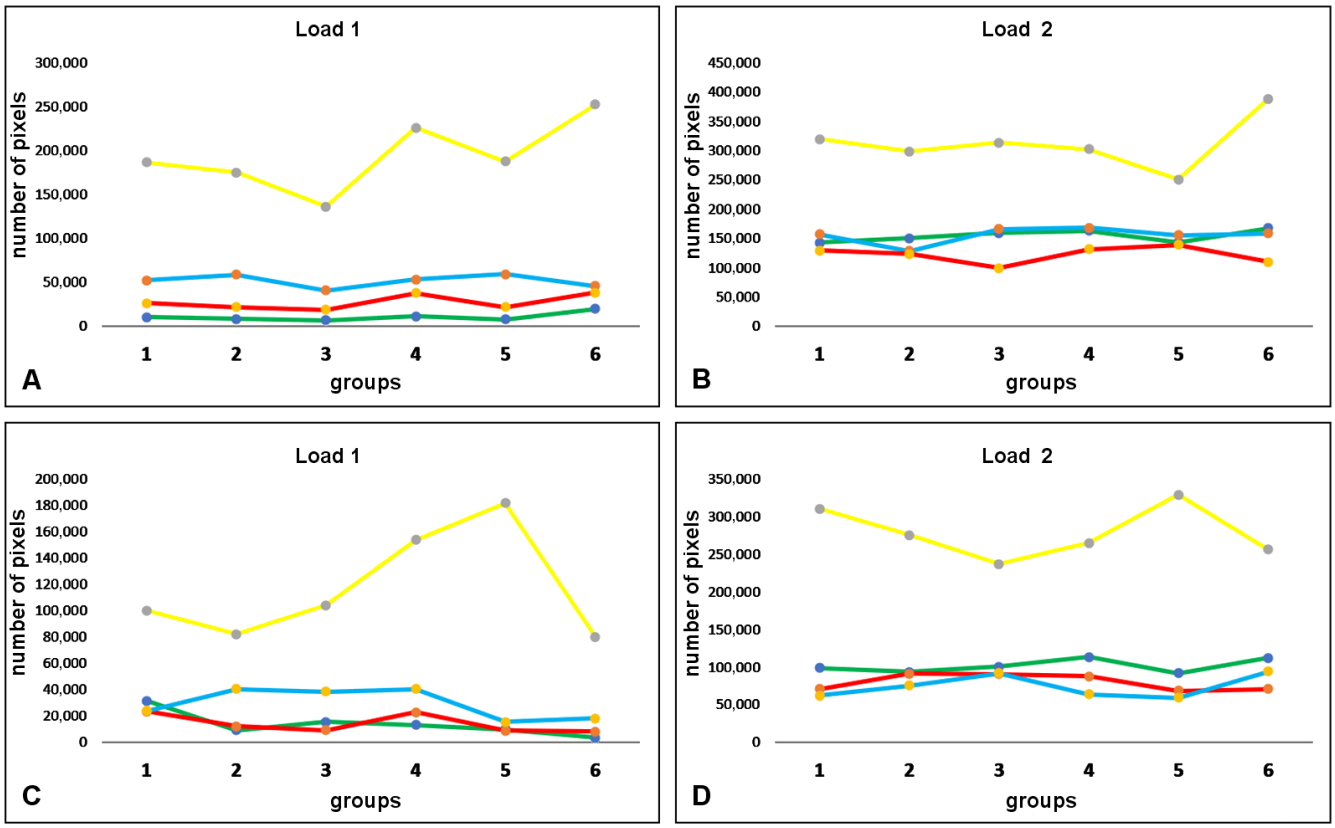

Fig. 11. Line graphs showing the distribution of the total number of pixels measured for each color in both image sizes
A, B – total images; C, D – crestal images.

Thus, the number of pixels of the yellow tone was determined to assess the variation in strain distribution between the groups. Then, when analyzing the yellow tone, which was the one with the highest number of pixels and the greatest variation, in the total images with load 1, the difference between the highest value (G6) and the lowest value (G3) was 85.3%; for load 2, the difference between the highest value (G6) and the lowest value (G5) was 55.1%. In the evaluation of the crestal area for the yellow tone with load 1 (the highest value – G5 and the lowest value – G6), the difference was 126.4%; for load 2 (the highest value – G5 and the lowest value – G3), it was 38.9%. The number of pixels of the yellow tone was measured for each group for the 2 loads; the data is shown in Table 3.

Table 3. Number of pixels in each group for the 2 loads, in both image sizes (values considering the yellow tone)

Group	Total images		Crestal images	
	load 1	load 2	load 1	load 2
G1	186,663	320,347	100,383	310,627
G2	175,520	298,863	82,431	275,527
G3	136,508	314,268	104,363	237,167
G4	226,105	302,810	153,995	265,357
G5	188,005	250,920	181,837	329,477
G6	252,983	389,077	80,299	256,602

Discussion

The application of ceramic materials in dental surgery and maxillofacial rehabilitation has increased significantly in recent years, and is receiving great attention from the scientific community.[17,18] The use of metal-free restorations has become popular in modern dentistry, mainly due to the improved cosmetic effect. In that sense, various materials with different chemical and mechanical characteristics have emerged. In the present study, 6 different materials used for the elaboration of single restorations were tested: feldspathic ceramic; metal-ceramic; polymer-filled ceramic; ceramic-stratified zirconia; monolithic zirconia; and lithium disilicate. The results revealed different behaviors in terms of strain distribution in the supporting structures under 2 load intensities applied to the samples. Thus, the initial hypothesis that there would be no important differences in the distribution of strain in the supporting tissues was discarded.

Several studies have shown that the use of conical connection implants (Morse-tapered implants) with switching platforms can contribute to reducing stress around the cervical portion of the implant.[19–22] Additionally, other studies have shown that Morse-tapered implants should be installed at a subcrestal level, which can improve the dissipation of stress in the bone tissue around the implants.[23,24] Therefore, in the present study, we opted for a Morse-tapered connection implant, positioning it at a subcrestal level of 2 mm.

In another finite element analysis study evaluating the influence of different types of cement on the distribution of stress in monolithic zirconia restorations, it was concluded that cements with a lower modulus of elasticity, such as resinous cements, distribute stress better than cements with a higher modulus of elasticity, like zinc phosphate-based cements.[25] For this reason, in the present study, a resinous cement was used to cement the crowns. Rungsiyakull et al. compared in a finite element analysis study the pattern of load distribution in parts with different cusp inclinations and at different points of force application, and concluded that both the cusp inclination and the point of force application had a significant influence on the transmission of loads to the supporting structures.[26] For this reason, in the present study, the loads were applied in the center of the crown, thus avoiding any possible variation due to differences in the anatomy of some of the cusps between the samples of each group.

Studies have shown contradictory results regarding the transmission of loads toward the supporting tissues by the different restorative materials used for crown manufacture.[27–30] Menini et al. performed a simulation in order to measure the occlusal forces transmitted by different materials to the peri-implant bone tissue and concluded that the use of softer materials, such as resin or acrylic, reduces the forces by up to 70.8% and 95.6%, respectively.[27] However, in the present study, only materials for permanent restorations were compared, and the results showed a difference of 75.5% between the highest and the lowest values of the area of strain distribution around the implant with load 1, and 19.8% with load 2. The G6 crowns made of lithium disilicate presented the highest strain values in terms of area and number of pixels of the colors for both applied loads. Other authors compared stress distribution in lithium disilicate ceramic and other ceramic materials, and reported that lithium disilicate ceramic crowns showed higher stress values under vertical loading.[31,32]

The loads received by the crown–abutment–implant sets during chewing are dissipated in greater intensity in the first millimeters of the interface between the bone tissue and the implant.[33,34] For this reason, this area has received a lot of attention from the scientific community. The thickness of the cortical bone as well as the quality of this tissue can directly influence the pattern of strain distribution. The cortical bone tissue, as it presents different characteristics and mechanical behavior in comparison with the medullary bone, mainly in terms of modulus of elasticity and viscosity, absorbs a greater amount of strain.[35] For this reason, the crestal area of load dissipation in each group was analyzed separately. The results of the evaluation of the crestal images showed a significant difference between the groups, being that for load 1, the highest concentration of strain was observed in G4 (zirconia covered with ceramic), while for load 2, the highest concentration of strain was noted for G5 (the crown made of zirconia – Y-TZP). Corroborating results have been reported in other studies that compared different restorative materials.[36]

However, in a finite element analysis study, Assunção et al. showed that when combining different materials on the one hand and varying the fit of the restorations on the other, the different hardness of the materials did not affect stress distribution, but it did increase strain on the implant and the screws of the restorations.[30] Other authors carried out an in vitro study to evaluate the wear of the tooth enamel produced by monolithic zirconia, lithium disilicate and composite, and reported that the wear produced by zirconia was similar to that produced by composite, but lower than in the case of lithium disilicate.[6] Yet another study looked for ceramic materials with the wear values similar to that of human enamel.[37] The wear produced by antagonistic teeth on human enamel and on different ceramics was evaluated. The authors concluded that leucite-reinforced glass, lithium disilicate glass and feldspathic porcelain had the wear values closer to that of human enamel as compared to yttria-stabilized zirconia.[37]

Factors such as implant macrogeometry, prosthesis design, material used, location and position of the implant as well as quantity and quality of the bone tissue directly influence the distribution of stress.[38–40] Strain around the implant depends on the implant–bone interface and is influenced by its biomechanical aspects, such as

the modulus of elasticity of the bone, the percentage of bone–implant contact (BIC), the spatial location of BIC, bone density, the degree of bone–implant bonding, etc.[41] Thus, strain in the peri-implant bone area is an important factor to be evaluated, as it results in bone stress.[42]

The photoelasticity test is commonly used in engineering. This type of analysis, which generates fringes with different colors, has been widely used also in dentistry for determining the pattern of stress dissipation in structures that receive loads. However, there is significant variation in the methodologies applied to evaluate the results. In some studies, the fringes were evaluated, in others – the dissipation area, and yet others proposed the development of computer programs for the determination and analysis of the stress dissipation values.[19,33,43,44] In the present study, we proposed a new methodology for data evaluation, in which it was possible to find a pattern of stress distribution after load application to determine differences in behavior between the various materials tested. The variation found in the pattern of load distribution around the implant indicated the yellow color as the main element of analysis. However, new studies must be carried out to prove and corroborate these findings.

Limitations

As a limitation of this study, we can report that the loads were applied with an almost quasi-static movement in a single direction, and at only one position of the crown. Another limitation is the fact that resin blocks have the same density throughout the human body; thus, differences between the portions corresponding to the cortical and medullary bones could not be shown. Some differences in the shape and morphology of the crowns, especially those that received the manual addition of ceramics, should also be considered as a limitation of this study. Finally, the use of only one sample could be considered a limitation; however, most photoelasticity studies were conducted in this manner.[19,33,43]

Conclusions

Within the limitations of this study, differences in stress distribution were observed among the 6 materials tested. The greatest values were detected in the crowns made of zirconia and lithium disilicate. In assessing stress dissipation in the crestal region of the implant, the zirconia crowns showed the highest values in comparison with other groups.

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Evaluation of the shear bond strength of zirconia to a self-adhesive resin cement after different surface treatment

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Abstract

Background. Bond stability between zirconia and a self-adhesive resin cement is a major concern, and only limited evidence about its longevity is currently available. Moreover, no study has yet comprehensively evaluated the influence of different power levels of the neodymium-doped yttrium-aluminum-garnet (Nd:YAG) laser irradiation on the shear bond strength (SBS) of zirconia to a self-adhesive resin cement.

Objectives. The aim of this study was to evaluate the SBS of pre-sintered and sintered zirconia to a self-adhesive resin cement after various treatment (air abrasion and the Nd:YAG laser irradiation at varying power levels – 1 W, 2 W and 3 W).

Material and methods. Ninety-nine zirconia specimens were prepared and divided into 3 groups: control (with no surface treatment); and pre-sintered and sintered groups with surface treatment. Surface treatment was applied before sintering in the pre-sintered group and after sintering in the sintered group. After following all protocols, a resin cement was layered on the zirconia surface. Shear bond strength was measured using a universal testing machine. The results were subjected to the statistical analysis. The surface topography and phase transformation of zirconia were evaluated using the atomic force microscopy (AFM), scanning electron microscopy (SEM) and X-ray diffraction (XRD) analyses after surface treatment.

Results. The laser irradiation (3 W, 1 W and 2 W) of the pre-sintered zirconia surface resulted in the highest SBS values ($p < 0.001$), while the lowest SBS values were obtained with airborne particle abrasion of the pre-sintered and sintered zirconia surfaces.

Conclusions. Laser irradiation increased the SBS of pre-sintered zirconia to a resin cement. Surface treatment with air abrasion had a lesser effect on the SBS values.

Keywords: shear bond strength, zirconia, resin cement, surface treatment, Nd:YAG laser

Introduction

Zirconia, especially an yttrium-stabilized tetragonal zirconia polycrystalline (Y-TZP) ceramic, is a durable and commonly used dental material that was introduced for single crowns,[1] frameworks for fixed partial dentures[1,2] and implant abutments[3] with the development of the computer-aided design/computer-aided manufacturing (CAD-CAM) technology. It can be used both in the anterior and posterior regions due to its favorable mechanical properties (high fracture toughness (7–10 MPa) and high flexural strength (700–1,200 MPa)) and color.[1–3] Zirconia can be used in a monolithic form or can be veneered using feldspathic porcelain.

Zirconia is a polymorphic material. When stimulated, it responds through a phase transformation mechanism, changing from its tetragonal (t) to monoclinic (m) phase, which results in superficial compressive stress with a grain volume increase of 3–5%. This prevents the further propagation of cracks and increases the toughness of zirconia.[4]

With reliable chemical bonding between a resin cement and a restorative material, more tooth structures can be preserved and more durable restorations with short clinical crowns can be performed.[5] A strong bond depends on many factors, such as surface roughness, wettability of the resin cement, and bonding ability of the cement and its chemical ingredients in particular.[6] It has been stated that the application of the 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) monomer containing a bonding agent is the key factor for a durable bond between zirconia and the tooth, and that the bonding agent improves the wettability of the treated zirconia surface.[7] Several studies have been conducted on the effect of different zirconia surface treatment on the quality of the resin cement–zirconia bond.[8–10] Common treatment options include the application of mineral acids, such as hydrofluoric acid and phosphoric acid, selective infiltration etching, experimental treatment with a hot-etching solution, and plasma spray treatment.[9,11,12] Hydrofluoric acid has the ability to remove the glass phase of the ceramic structure in a process that produces a micro-retentive surface with high free energy, which increases the adhesion between the resin cement and the ceramic through the application of a silane agent. However, acid etching is not suitable for zirconia, as it does not have a glassy phase.

Various mechanical treatment techniques, including air abrasion with Al_2O_3 particles of the preferred size range of 25–250 μm and laser surface treatment, such as irradiation with the neodymium-doped yttrium-aluminum-garnet (Nd:YAG), erbium-doped yttrium-aluminum-garnet (Er:YAG), erbium, chromium-doped yttrium-scandium-gallium-garnet (Er,Cr:YSGG), and carbon dioxide (CO_2) lasers, have also been applied and evaluated for improving the resin cement–zirconia adhesion.[6–9]

Air abrasion with Al_2O_3 particles combined with a 10-MDP-containing primer/resin cement has been suggested as an effective technique for bonding.[7] However, it has been criticized for causing micro-cracks within zirconia by increasing the content of the m phase,[6,9,12] and additional heat treatment would be required to decrease the m phase.[10] As an alternative, some researchers proposed different kinds of pretreatment of the pre-sintered zirconia surface[5,10,13] and found that the value of the zirconia shear bond strength (SBS) significantly increased after the air abrasion treatment.[5] Additionally, the air abrasion of pre-sintered zirconia followed by sintering could provide some advantages, such as a higher surface roughness with deep crevices and projections, which increases the content of the t phase, and no additional heat treatment but air abrasion is needed after the sintering procedure.[10] Although the air abrasion technique remains questionable, it is still regarded as effective.

Meanwhile, various lasers have been applied to achieve increased surface roughness, wettability and SBS of zirconia in its sintered form. It has been reported that the Nd:YAG, Er:YAG and Er,Cr:YSGG lasers could help to produce a strong bond at the resin cement–zirconia interface.[4,14] Among the 3 effective lasers (Nd:YAG, Er:YAG and Er,Cr:YSGG), the highest surface roughness of zirconia has been recorded with the use of the Nd:YAG laser.[9] Gandolfi Paranhos et al. reported that they achieved a higher surface roughness of zirconia with the Nd:YAG laser as compared to the CO_2 laser.[15] However, some researchers reported that lasers might induce phase transformation through a high laser power and uncontrolled temperature, which may also damage the zirconia surface.[9,16,17] The use of laser irradiation for the treatment of the pre-sintered zirconia surface is an alternative to the treatment of sintered zirconia, and it also helps to improve SBS at the resin cement–zirconia interface.[13,14] It has been previously reported that prolonging the irradiation time in addition to increasing the irradiation power does not increase the adhesion strength of ceramics and causes defects in the material.[18] For this reason, the questions how much irradiation power is used and for how long the zirconia material is irradiated are important in terms of SBS of the material and defects that may occur in it. In this study, the Nd:YAG laser was used, as it increases the surface roughness and wettability of zirconia, and thus a high value of SBS can be achieved.

Several kinds of surface treatment of pre-sintered or sintered zirconia have been recommended to improve the SBS of zirconia to a resin cement. However, the effect of laser treatment at varying power levels on the SBS of zirconia in different forms to a resin cement is not well known. The aim of this in vitro study was to evaluate the effect of different surface treatment (airborne particle abrasion and the Nd:YAG laser irradiation at varying power levels) on the SBS of pre-sintered or sintered zirconia to a resin cement. The null hypothesis was that the zirconia form and surface treatment would not affect the resin cement–zirconia SBS values. Also, the surface topography and phase transformation of zirconia were evaluated using the atomic force microscopy (AFM), scanning electron microscopy (SEM) and X-ray diffraction (XRD) analyses.

Material and methods

Sample preparation

Ninety-nine pre-sintered zirconia specimens (Kuraray Noritake Dental Inc., Nagoya, Japan), 7 mm in diameter and 3 mm in height, were prepared using a CAD-CAM milling device (Yenamak D50; Yenadent, Istanbul, Turkey). Of those, 9 specimens were used for the AFM, SEM and XRD analyses. A grinding machine (Phoenix Beta Grinder/Polisher; Buehler, Esslingen am Neckar, Germany) was used with 600-, 800- and 1,200-grit silicon carbide abrasives (English Abrasives & Chemicals Ltd., Stafford, UK) for 15 s to form a standard bonding surface on the zirconia specimens. A flowchart of the test protocol used in the study is illustrated in Fig. 1.

Forty specimens were sintered at 1,500°C for 8 h, according to the manufacturer's recommendations (the sintered group). The specimens were divided into 3 groups: pre-sintered control (pre-sintered-C); pre-sintered; and sintered. The pre-sintered and sintered groups were then divided into 4 subgroups according to the surface treatment technique. Surface treatment and bonding procedures were performed by the same operator. The control group specimens received no further surface pretreatment. The airborne particle abrasion subgroups consisted of pre-sintered and sintered zirconia specimens that were abraded with 120-micrometer Al_2O_3 particles at a pressure of 200 kPa for 20 s at a distance of 10 mm. The laser subgroups contained specimens that were irradiated with the Smarty A10 Nd:YAG laser (DEKA Laser, Calenzano, Italy), which produces laser pulses at a wavelength of 1.06 µm. The optical fiber of the laser was 300 µm in diameter and was held manually perpendicular to the zirconia ceramic for 20 s at a distance of 1 mm, as described in previous studies.[7,8] The laser parameters included a pulse energy of 100 mJ, a repetition rate of 10, 20 and 30 Hz, and a pulse duration of 320 µs (Table 1).

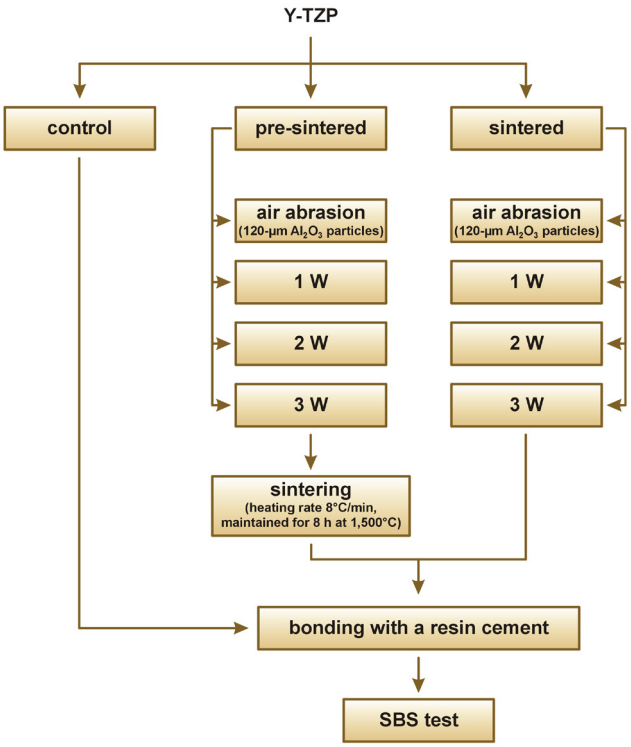

Fig. 1. Flowchart of the test protocol used in the study

Y-TZP – yttrium-stabilized tetragonal zirconia polycrystalline; SBS – shear bond strength.

Table 1. Laser parameters used in the study

Parameter	Nd:YAG laser characteristics
Manufacturer	DEKA Laser, Calenzano, Italy
Model identifier	Smarty A10
Wavelength	1,064 nm
Delivery system	300-micrometer quartz optical fiber
Power	1 W, 2 W, 3 W
Power density	1.4 W/cm^2
Energy density	85 J/cm^2
Duration	20 s at a distance of 1 mm
Repetition rate	10 Hz, 20 Hz, 30 Hz
Pulse duration	320 µs

Nd:YAG – neodymium-doped yttrium-aluminum-garnet.

Shear bond strength test

All pre-sintered specimens were sintered at 1,500°C for 8 h again. A self-adhesive dual-polymerizing resin cement (Bifix SE, Lot: 1824158; VOCO, Cuxhaven, Germany) was applied to both the untreated and treated bonding surfaces with the use of cylindrical Tygon® tubes, 3 mm in diameter and 3 mm in height, in accordance with the manufacturer's recommendations. The Tygon tubes were then removed and all resin cement–zirconia specimens were immersed in distilled water at 37°C for 72 h before testing SBS. Using a crosshead speed of 1 mm/min, each specimen was loaded to fracture in a universal testing machine (Lloyd LF Plus; AMETEK, Meerbusch, Germany) for the SBS test.

AFM analysis

After surface treatment, 1 randomly selected specimen from each group was studied by means of an AFM (Park Systems, Suwon, South Korea). The AFM was used in the non-contact mode with a cantilever (NSC 36; Micro-Masch, Tallinn, Estonia) with an aluminum-doped silicon tip (a radius of curvature of 10 nm or less). The measurements were performed with the aid of a computer program (XEP software; Park Systems). The grains were measured over an area of 1 × 1 µm. All images of 512 × 512 px were obtained with a low scanning frequency (0.3 Hz) from each surface.

SEM analysis and the fracture pattern analysis

One specimen was selected from each group and analyzed with an SEM (JSM-6060LV; JEOL Ltd., Tokyo, Japan) at ×500 and ×1,000 magnification after being coated with gold-palladium. After the SBS test, the fracture patterns were detected using a stereomicroscope (Stemi™ DV4; Carl Zeiss Microscopy, Göttingen, Germany) at ×32 magnification. The fracture patterns were classified as adhesive failure (at the interface between the zirconia surface and the cement), cohesive failure (within the cement) or mixed failure (adhesive and cohesive).

XRD analysis

The XRD patterns of 1 sample from each group were obtained with an X-ray diffractometer (Empyrean; Malvern Panalytical Ltd., Malvern, UK) using Cu Kα radiation (λ = 1.5406 Å; 45 kV and 40 mA). The calculations were based on the Garvie and Nicholson method,[19] which is a currently accepted one.

Statistical analysis

In order to analyze differences in the SBS values between the groups, the one-way Welch *F* test (without assessing the homogeneity of variance) and the Games–Howell post-hoc test were used. The statistical analysis was performed using the IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA), with a significance level at $p < 0.05$.

Results

Shear bond strength was significantly affected by the zirconia form (pre-sintered or sintered) and by surface treatment (Table 2). The SBS values for each group with different surface treatment are displayed in Table 3. The SBS values obtained with laser irradiation at power levels of 1 W, 2 W and 3 W on the pre-sintered zirconia surface were significantly higher as compared to the untreated surface and the sintered subgroups. Laser irradiation at varying power levels slightly increased the SBS values of the sintered zirconia surface with regard to the air abrasion sintered subgroup except for the 3 W subgroup. However, there were no significant differences with regard to the control group. The laser irradiation pre-sintered subgroups showed the highest SBS values (18.3 ±2.7 MPa (3 W), 14.1 ±2.8 MPa (1 W) and 13.1 ±2.7 MPa (2 W)) ($p < 0.001$). The lowest SBS values were obtained with airborne particle abrasion in the case of both pre-sintered and sintered forms.

The analysis of failure revealed that fracturing occurred predominantly at the resin cement–zirconia interface (48.88%). Cohesive failure in the resin cement was infrequent (20.00%) and mixed failure (adhesive and cohesive) occurred with a moderate frequency (31.12%).

Table 2. Results of the one-way Welch *F* test with regard to the shear bond strength (SBS) values [MPa] in all groups

Statistical test	df1	df2	*F*	*p*-value
Welch *F* test	8	33.119	42.610[a]	0.000*

df – degree of freedom; [a] asymptotically *F*-distributed; * statistically significant.

Table 3. Shear bond strength (SBS) values for all groups ($n = 11$)

Groups	SBS [MPa]
Control	6.0 ±1.1[a,c]
Air abrasion pre-sintered	4.5 ±1.1[a,d]
Air abrasion sintered	4.7 ±0.9[a]
1 W laser irradiation pre-sintered	14.1 ±2.8[b,e]
1 W laser irradiation sintered	6.8 ±0.5[c]
2 W laser irradiation pre-sintered	13.1 ±2.7[b]
2 W laser irradiation sintered	6.1 ±0.7[c,d]
3 W laser irradiation pre-sintered	18.3 ±2.7[e]
3 W laser irradiation sintered	6.0 ±1.3[a,c]

Data presented as mean ± standard deviation (*M* ±*SD*). The same letters in superscript show no statistically significant difference ($p > 0.05$).

The relative amount of monoclinic zirconia which was defined with the XRD analysis on the untreated and treated surfaces (pre-sintered and sintered) of all specimens are presented in Fig. 2. Nearly 100% t phase was obtained on pre-sintered and sintered zirconia before surface treatment. After mechanical treatment (airborne particle abrasion or laser irradiation), there was an increase in the m content of zirconia, especially in the pre-sintered subgroups. After the sintering process was performed in the pre-sintered subgroups, the m peaks disappeared and the m content was similar to that of the untreated surface (control group).

The SEM and AFM analyses revealed that the zirconia surface was modified after different treatment. While there were more homogeneous layers formed on the airborne particle-abraded (pre-sintered and sintered) surfaces, which did not show any major morphological differences as compared to the untreated surface (Fig. 3), the laser-irradiated pre-sintered surfaces were irregular, with melting and the formation of pits (Fig. 4). The laser irradiation of the pre-sintered specimens led to the formation of well-defined micro-sized elevated and depressed areas, which was possibly due to the high impact of the laser power (Fig. 4). Nevertheless, the treatment of the sintered surfaces with laser irradiation resulted in wide carbonization areas with macro-cracks as compared to the control group (Fig. 5).

The areas created on the pre-sintered surfaces by laser irradiation could provide retention for the bonding of the resin cement (Fig. 6).

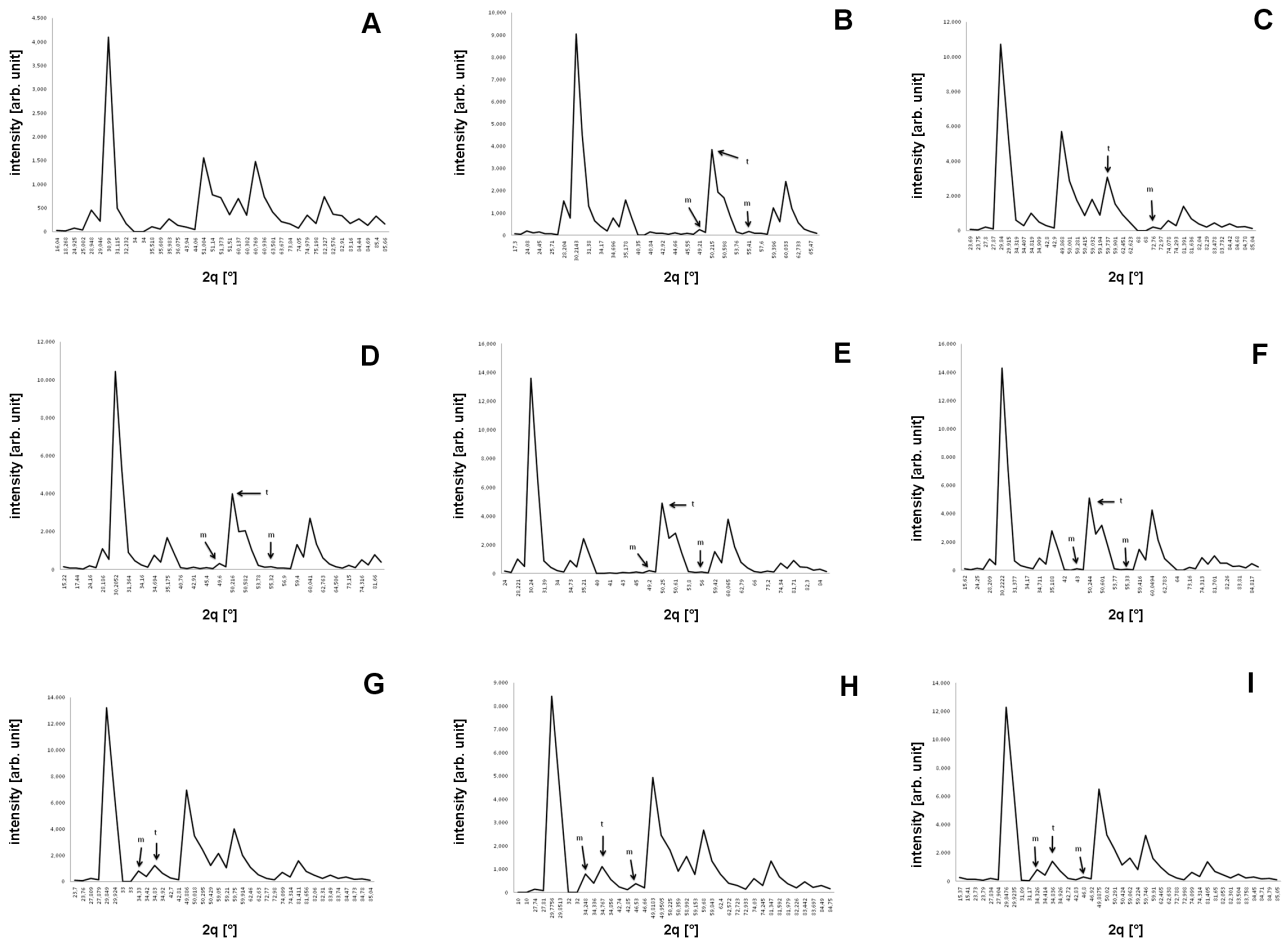

Fig. 2. Results of the X-ray diffraction (XRD) analysis of the pre-sintered and sintered zirconia surfaces

A – untreated pre-sintered; B – air-abraded pre-sintered; C – air-abraded sintered; D – laser-irradiated (1 W) pre-sintered; E – laser-irradiated (2 W) pre-sintered; F – laser-irradiated (3 W) pre-sintered; G – laser-irradiated (1 W) sintered; H – laser-irradiated (2 W) sintered; I – laser-irradiated (3 W) sintered.

Sintered zirconia had a rougher surface than the untreated pre-sintered zirconia (Fig. 3A, 3B). It was observed that the sandblasting of sintered zirconia provided a rougher surface as compared to the sandblasting of pre-sintered zirconia (Fig. 3D–G). The laser irradiation of the pre-sintered and sintered zirconia surfaces resulted in rougher surface textures as compared to the groups without laser irradiation (Fig. 3–5).

Discussion

The null hypothesis of this study was rejected, since the treatment of pre-sintered zirconia affected the SBS of zirconia to the resin cement. In addition, the mechanical treatment (airborne particle abrasion and laser irradiation at different power levels) of zirconia increased the transformation of the m phase.

The accurate measurement of bond strength at the zirconia–veneer porcelain interface is quite complex. However, the SBS test is a common method applicable to zirconia-based ceramic systems.[20] As most of the stresses related to the fracture of the bond between the tooth and the restoration are the stresses of shearing in clinical terms,[21] and since the applied forces are perpendicular to the bond surface, the small cross-sectional area of the bond surface virtually eliminates the combination of structural defects that significantly affect the test readings.[22] Therefore, in this study, the SBS test was used to assess the SBS of zirconia to the resin cement. Consequently, no further specimen processing was required after the bonding procedure.

In addition to obtaining a reliable bond between zirconia and a resin cement, the purpose of the zirconia surface treatment is to increase the surface area, which can result in improved wettability.[7,8] A proportionally larger area has a high surface energy, which can improve mechanical bonding to the zirconia surface.[9,18] Airborne particle abrasion has gained popularity with regard to obtaining roughness on the zirconia surface,[23] but conflicting results were reported in some related studies.[8,24,25]

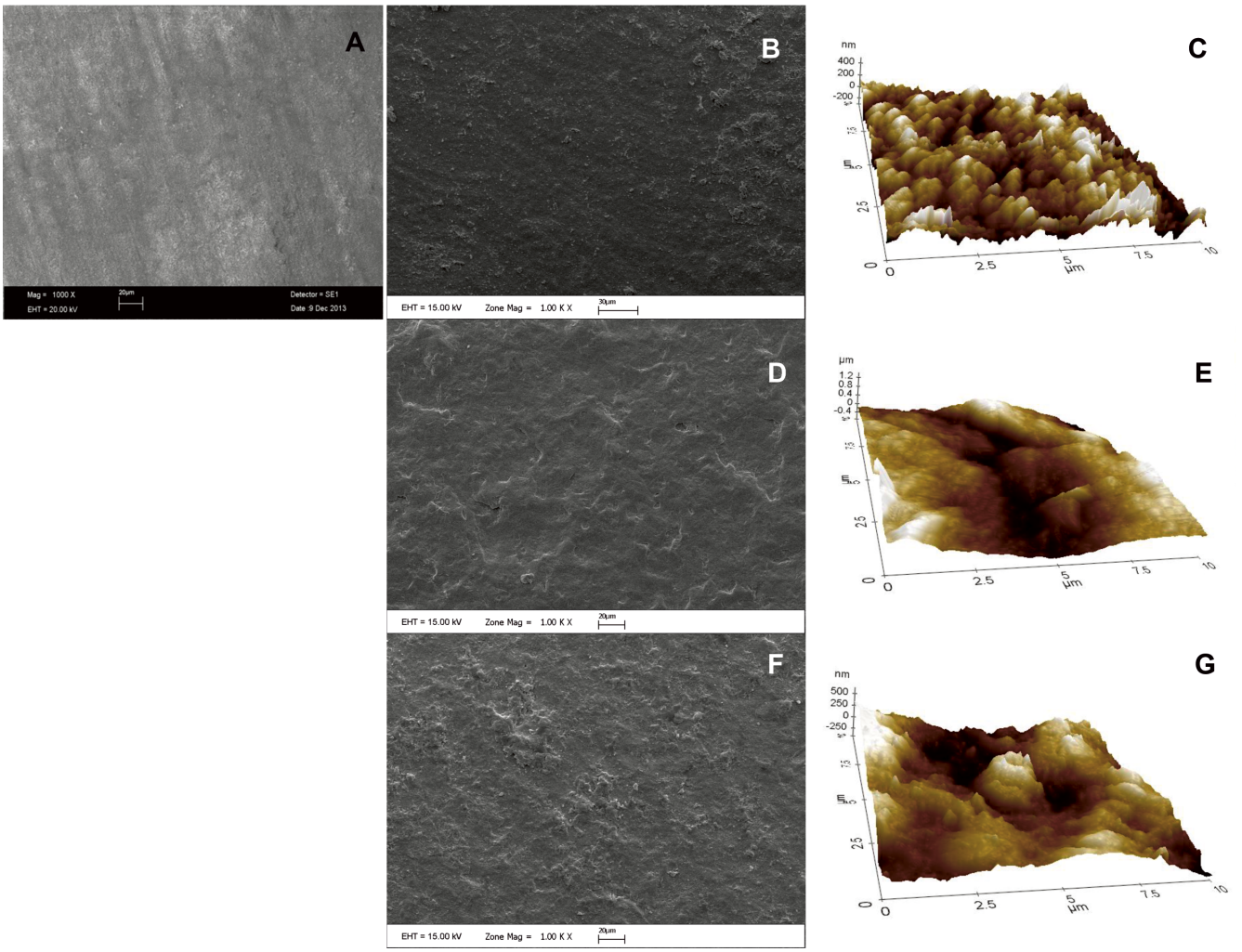

Fig. 3. Scanning electron microscopy (SEM) image of the untreated pre-sintered zirconia surface (A), and the SEM and atomic force microscopy (AFM) images after sintering (B, C), the air abrasion of the pre-sintered zirconia surface (D, E) and the air abrasion of the sintered zirconia surface (F, G) magnification ×1,000.

In many studies applying surface treatment, where SBS and surface roughness were evaluated, there was no correlation between the SBS values and the surface roughness values.[17,26] A strong bond depends on many factors, such as roughness, wettability of the resin cement, cementation with self-, light-, or dual curing, and composition of the materials. Roughness is just one of them.

The application of sandblasting has the disadvantage of slightly changing the surface chemistry due to the alumina contamination it causes.[27] The roughening of the zirconia surface with the airborne particle abrasion method has been suggested because of the absence of polycrystalline and glass phases on the zirconia surface, unlike in the case of glass-ceramics.[8] Some manufacturers recommend the airborne particle abrasion of the zirconia surface before cementation as a routine surface treatment technique. In a previous study, the application of airborne particle abrasion with 50-micrometer Al_2O_3 particles to sintered zirconia enhanced the interfacial SBS.[14] It has been suggested that increasing the irregularities on the airborne particle-abraded zirconia surface may enhance the strength of the bond.[14] Several studies reported similar results, with a 10-MDP-containing resin cement providing the highest SBS values when used on the zirconia surface treated with airborne particle abrasion with 50-micrometer alumina particles.[9,10] Contrarily, the SBS values of the airborne particle abrasion group which was treated with 120-micrometer alumina particles were the smallest among all test groups in the current study. This indicates that the airborne particle-abraded surface may provide an unsatisfactory resin cement–zirconia bond. This is supported by the SEM and AFM images, which revealed no effect of airborne particle abrasion in modifying the zirconia surface, i.e., the surface was relatively smooth (Fig. 3D–G). Additionally, for the specimens treated with airborne particle abrasion (sintered and pre-sintered), adhesive failure was observed in most of the debonded specimens. Some kinds of mechanical treatment (airborne particle abrasion, laser irradiation, etc.) may damage the zirconia surface by increasing the content of the m phase. In the current study, it was also found that airborne particle abrasion resulted in the transformation of the m phase (6.6%). Recently, some researchers have

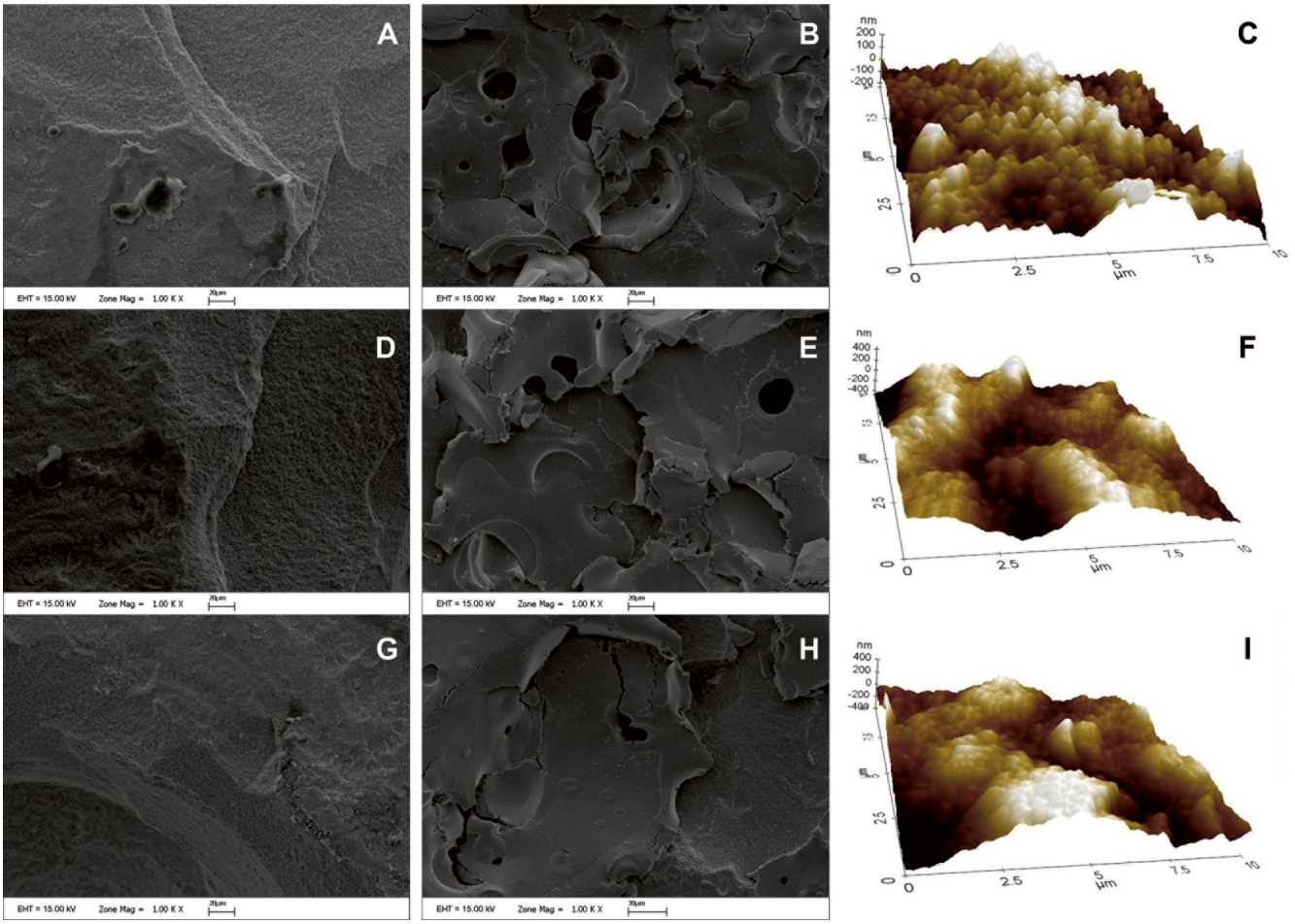

Fig. 4. Scanning electron microscopy (SEM) images of the laser-irradiated pre-sintered zirconia surfaces (A – 1 W; D –2 W; G – 3 W), and the SEM and atomic force microscopy (AFM) images after sintering (B, C – 1 W; E, F – 2 W; H, I – 3 W)
magnification ×1,000.

started using the airborne particle abrasion treatment of the pre-sintered zirconia surface to increase retention to zirconia.[10,13,14] However, it is believed that airborne particle abrasion generates stress on the pre-sintered zirconia surface and increases the content of the m phase. Thus, the values of the m phase dropped to almost zero after sintering in the present study. This might be explained by the regeneration firing procedure, which induced the m–t phase transformation. In addition, in the laser irradiation 1–3 W pre-sintered subgroups (Fig. 2D–F), XRD patterns revealed a lower m phase than in other laser irradiation subgroups. The m–t phase transformation occurred during the sintering process and the final proportion of the m phase was almost zero in the pre-sintered laser irradiation subgroups, but 38.8%, 51.5% and 44.7% in the laser irradiation sintered subgroups, respectively for 1 W, 2 W and 3 W.

To date, numerous studies have been published about the effect of laser treatment on the SBS of zirconia to a resin cement or a veneer ceramic as well as on the roughness of zirconia.[8–10,14,17] Laser etching is a simple and effective technique for surface treatment. It removes organic and inorganic tissue particles through a process called ablation of dental tissue and by melting the surface dental materials through the absorption of laser energy.[6] The present study revealed that the laser irradiation of sintered zirconia did not have a significant effect on the SBS of zirconia to the resin cement when compared to the untreated surface. This could be attributed to a high laser energy that caused the formation of cracks on the zirconia surface due to temperature changes, which can create internal tension during laser irradiation (Fig. 5A, 5C and 5E). Low energy levels had a less destructive effect on the zirconia surface, as the SBS values slightly increased in the 1 W and 2 W subgroups, and decreased in the 3 W subgroup. On the other hand, the results of the current study are in conflict with a previous one, where the Nd:YAG laser irradiation (a pulse duration of 200 mJ and a repetition rate of 10 Hz) enhanced the SBS of the resin cement–zirconia bond.[28] The researchers also reported that the relative m phase proportion of laser-irradiated zirconia specimens was 26.5% and 30.5% for a long and a short pulse duration, respectively. The difference was due to a longer irradiation time (60 s) and the fact that the surface of zirconia was coated with graphite.[28] Similarly, one study revealed that the Nd:YAG laser could enhance SBS.[1] In addition, with regard to the failure types at the resin cement–zirconia interface, 50% referred to mixed failure

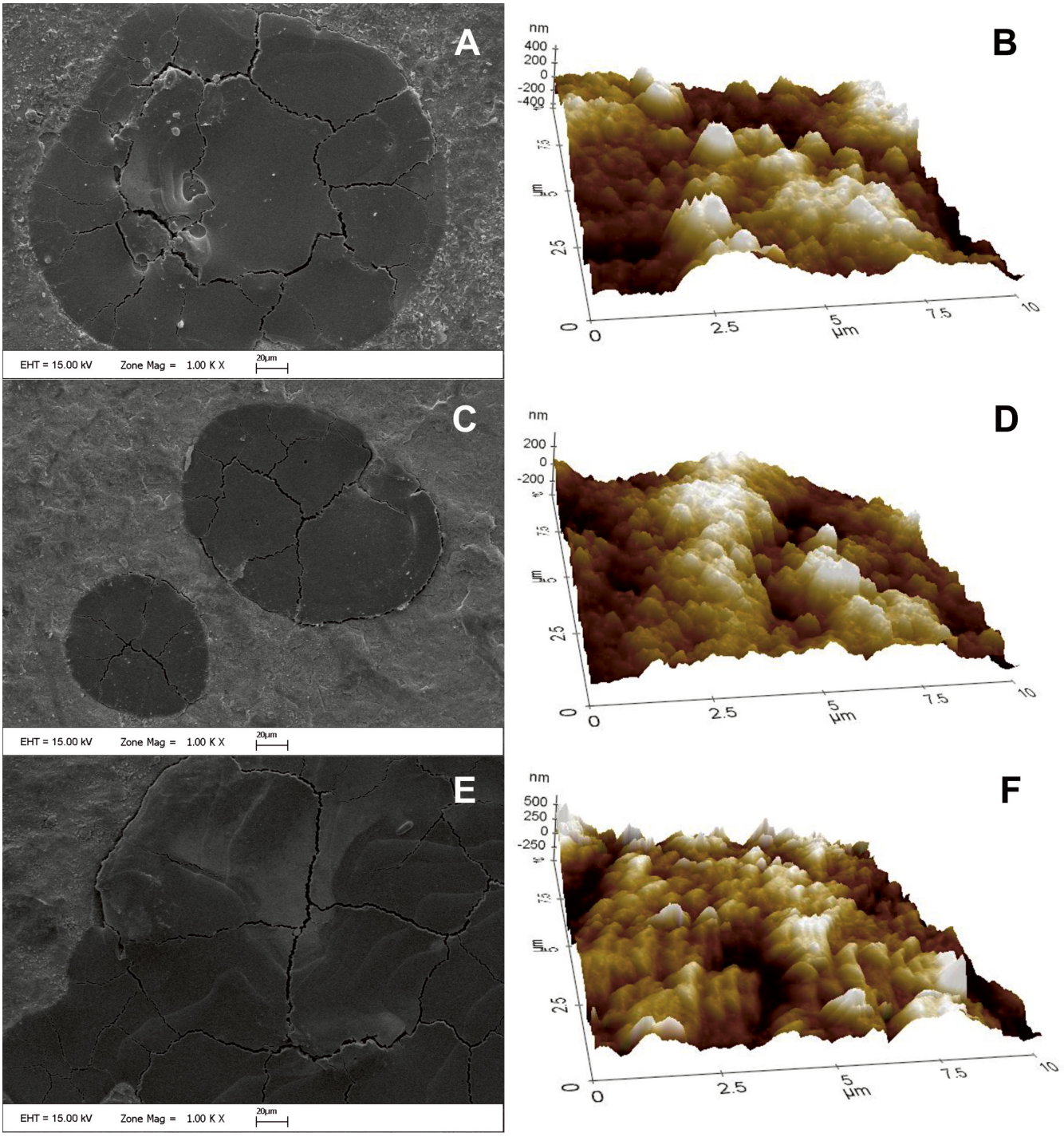

Fig. 5. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) images of the laser-irradiated sintered zirconia surfaces (A, B – 1 W; C, D –2 W; E, F – 3 W)
magnification ×1,000.

in the Nd:YAG laser group.[1] A previous study showed that a durable resin cement–zirconia bond was achieved after performing the Nd:YAG laser irradiation and using resin cements containing the 10-MDP monomer.[15] Further studies should be performed to develop new techniques with reliable procedures.

The m–t phase transformation enhances the fracture tendency in zirconia. Various kinds of mechanical treatment are responsible for triggering the transformation from the m to the t phase and regeneration firing should be done.[10] The airborne particle abrasion of pre-sintered zirconia in advance, and then performing the sintering procedure increased the m–t phase transformation by creating a retention area on the surface.[10] The effect of laser irradiation on the pre-sintered zirconia surface has been reported and the values of zirconia SBS have been stated to be significantly increased after irradiating zirconia with various lasers.[13,14] Such changes in the

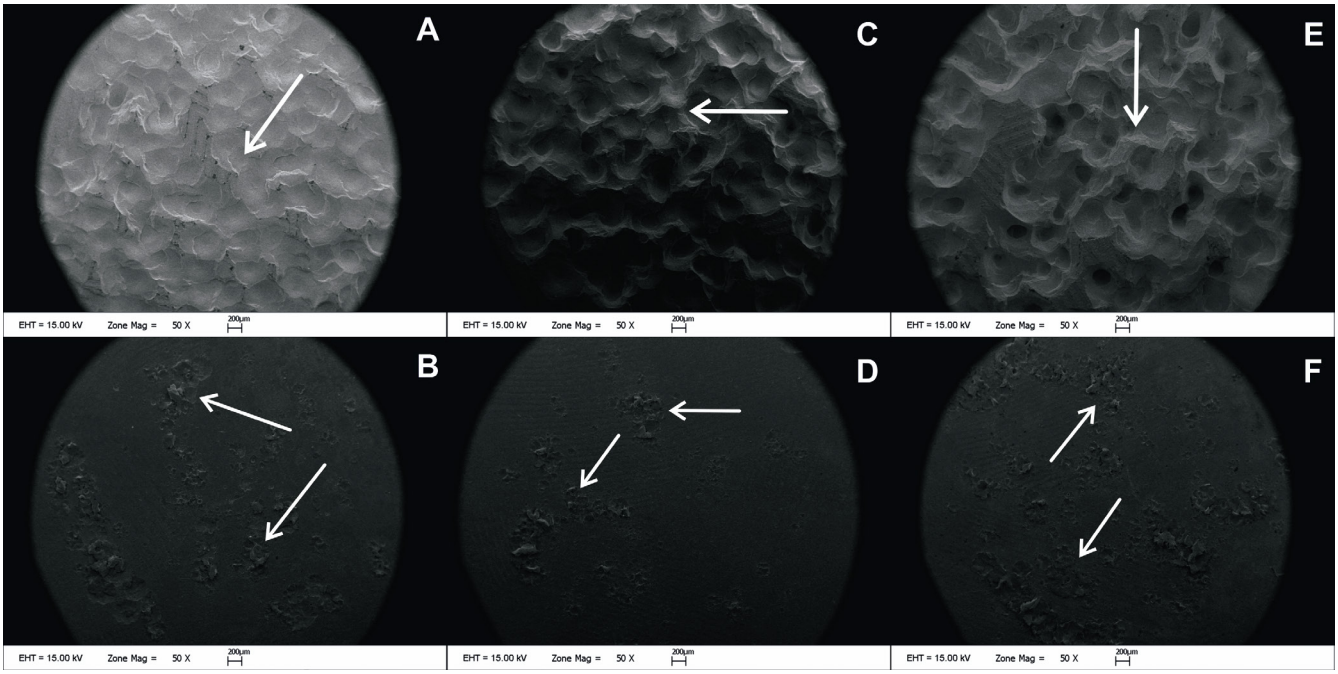

Fig. 6. Scanning electron microscopy (SEM) images of the pre-sintered zirconia surface after laser irradiation at 1 W (A) and after sintering (B), of the pre-sintered zirconia surface after laser irradiation at 2 W (C) and after sintering (D), and of the pre-sintered zirconia surface after laser irradiation at 3 W (E) and after sintering (F)

Arrows indicate melting areas; magnification ×500.

morphology of the zirconia surface may consequently result in different SBS values. In the present study, it was found that the laser irradiation of the pre-sintered zirconia surface, which results in a chalk-like form that is not hard, formed a significantly greater retentive area (Fig. 6). This situation may have positively affected the SBS of zirconia to the resin cement. While the pre-sintered subgroups commonly presented a cohesive type of failure, other subgroups showed adhesive failure.

Although zirconia restorations are clinically successful,[29] there are conflicting reports in the literature regarding the safest surface treatment to be used to enhance the lifespan of such restorations.[7,10,13,14,17] Having that in mind, the authors of this study believe that there is a need for improvement of the SBS between cements and the zirconia surface, while maintaining the integrity of the zirconia surface itself. Accordingly, the zirconia form and surface treatment to prepare the restoration for cementation are important factors to be taken into consideration.

Limitations

The 1st limitation of the current study is that chemical bonds were not researched. A chemical bond, just as a mechanical bond, largely affects SBS. The chemical bond between the phosphate groups and zirconia has been previously suggested[30] and might be the reason for different results in each group of the present study. Stronger adhesion between zirconia and the resin cement was obtained with the treatment of the pre-sintered zirconia surface. Restorations are exposed to many environmental factors in the oral cavity, such as moisture, and mechanical and thermal fatigue. The 2nd limitation of this study is the absence of thermocycling. To simulate more realistic clinical conditions in in vitro studies, methods such as water storage, mechanical loading or thermocycling are used. Moreover, applying thermocycling or mechanical cycling in addition to thermocycling may be effective in obtaining more accurate results by simulating intraoral conditions. In addition to the effect of thermal cycles at 5°C and 55°C, further studies could focus on the efficiency parameters of various lasers for future development. Also, it would be of interest to evaluate the surface topography of zirconia after different pretreatment by using confocal microscopy with SEM and AFM.

Conclusions

Within the limitations of this in vitro study, the following conclusions can be drawn:

– the surface treatment of pre-sintered zirconia with the 1–3 W Nd:YAG laser irradiation enhanced the SBS of pre-sintered zirconia to the resin cement;
– the airborne particle abrasion treatment in the pre-sintered and sintered groups decreased the SBS of zirconia to the resin cement; and
– mechanical treatment generated stress on the zirconia surface and increased the t–m phase transformation; the m–t transformation occurs during the sintering process, when it is used after pre-sintering surface treatment.

Although laser application is expensive, the fact that a single laser device has many application options (caries removal, restoration removal, canal irrigation, dentin sensitivity treatment, etc.) increases the demand for lasers. This situation makes researchers conduct in vitro studies on additional procedures that can be performed with lasers. In this context, this study showed the parameters and forms at which the laser can be applied to increase the adhesion of zirconia to a resin cement in addition to its routine application. Laser irradiation at the tested power levels may be preferred by clinicians on pre-sintered zirconia for improving SBS between a resin cement and pre-sintered zirconia.

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Effect of dental implant angulation on the dimensional accuracy of master casts

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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. Making accurate impressions of dental implants and transferring their three-dimensional (3D) position to master casts is critical for the passive fit of prosthetic frameworks.

Objectives. This study aimed to assess the effect of dental implant angulation on the dimensional accuracy of master casts.

Material and methods. An acrylic model with 2 external hexagonal implants was used in this in vitro experimental study. The impressions of the model were made in 42 positions, with different angulation of the 2 implants, ranging from +15° to −15°, by means of the open-tray and closed-tray impression techniques, using a polyvinyl siloxane impression material. The spatial coordinates of the implants were measured on the X, Y and Z axes. The dimensional accuracy of the impressions made at different positions (parallel, convergent and divergent) and different angulation of the implants were determined. The data was analyzed using the one-way analysis of variance (ANOVA), Student's *t* test and Tukey's test.

Results. Casts with the lowest accuracy were obtained when the 2 implants were divergent by 25° (R = 1.1336). However, the position of the 2 implants had no significant effect on the dimensional accuracy of the master casts. The error rate was 0.4181 in the open-tray technique and 0.5095 in the closed-tray technique, with no significant difference between them ($p > 0.05$). The angulation of the 2 implants had a significant effect on the dimensional accuracy of the master casts ($p = 0.0001$).

Conclusions. Considering the significant effect of implant angulation in the range from +15° to −15° relative to the longitudinal axis on the dimensional accuracy of master casts, further studies are required to reach a final conclusion in this respect.

Keywords: dental implants, dimensional accuracy, dental impression technique, dental implant angulation, master cast

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Introduction

Dental implants are presently in high demand. Due to the high success rate of implant treatment, implant-supported restorations are the first choice for prosthetic treatment.[1] Biomechanically, implant-supported restorations are fabricated with the aim of achieving passive fit in the attachment of abutments and fixtures. Passive fit is imperative to ensure equal stress distribution at the bone–implant interface. Precise clinical and laboratory steps are taken to achieve passive fit.[2] Misfit would cause internal stresses, and their subsequent transfer to implants and the bone matrix.[3] In the fabrication of implant-supported restorations, the accurate transfer of the intraoral position of the implant to the cast is much more important than it is for natural teeth.[4] In these restorations, the applied physiological forces are transferred to the surrounding bone in a manner that is similar to what occurs in natural teeth.[5] However, due to the absence of the periodontal ligament around a dental implant, misfit can result in destructive stresses in the bone–implant complex.[3] Moreover, evidence shows that the absence of passive fit in implant-supported restorations can cause tiny bone fractures or ischemic marginal zones. The healing of these lesions involves the formation of fibrous connective tissue at the bone–implant interface and prevents osseointegration or causes peri-implant bone loss.[5]

Framework misfit can cause biological and biomechanical problems. The application of excessive load, exceeding the physiological threshold of the implant-supporting bone, can cause pain, tissue irritation, marginal bone loss, and impaired osseointegration.[4,6] The mobility and fractures of implant components are among biomechanical problems that may occur.[7,8]

Moreover, active fit is the primary cause of the mobility of restorations, abutment screw loosening, bone loss, and the fractures of implant components.[9] The amount of stress that can clinically compromise the long-term stability of implants has not been determined.[10] It seems that accurate impression making, the safe transfer of the impression to the laboratory without distortion and the fabrication of an accurate model are important to achieve passive fit.[11] The first step is to precisely record the position of the implant in the oral cavity and transfer it to the cast by making an accurate impression.[12] However, in all impression techniques, a range of errors may occur in the transfer of the implant position, and the elimination of errors in impression making is not clinically feasible.[13] As mentioned, impression making is an important step in the fabrication of implant-supported restorations. It plays a fundamental role in obtaining the dimensional accuracy of the final cast.[4,14] However, it should be noted that when the angulation or position of the implant are incorrect, the impression making process is difficult, and requires more time and higher precision.[15]

Considering the controversy regarding the dimensional accuracy of different impression techniques and the gap of information on the selection of the best impression technique for angulated implants, further studies on this topic seem imperative. This study aimed to assess the effect of dental implant angulation on the dimensional accuracy of master casts.

Material and methods

In this in vitro study, the impression of the mandible of a 53-year-old patient was made. The patient had implants at the sites of teeth 29 and 31. A special tray was custom-made for this patient. An accurate impression was made and a dental stone cast was fabricated. The master model was prepared by duplicating the stone cast and pouring an acrylic resin (Lucitone® Clear; Dentsply International, York, USA). At the art portion of the master model, 3 tray stops were fabricated. It should be noted that this model did not have teeth 17, 29, 30, 31, and 32, according to the Universal Numbering System. Also, at the sites of teeth 18, 19 and 28, metal balls with a diameter of 3 mm were incorporated, acting as reference points in measurements.

A metal device was designed to adjust the different angles of the impression copings. The device consisted of 2 stainless steel plates connected to each other by means of 3 vertical rods (Fig. 1). The upper plate measured 198 mm in length, 151.5 mm in width and 10.3 mm in height. The lower plate measured 197.5 mm in length, 151.2 mm in width and 18.73 mm in height. Two of the connecting rods located at the sides of the device had a diameter of 20 mm and a length of 163.5 mm, while the third rod was at the front, and measured 10 mm in diameter and 101 mm in length. In the lower plate, 2 holes with a diameter of 20 mm were made to pass the 2 side rods and connect them to the upper plate. A number 6 screw was used to tighten the rods. Both of the rods passed through the upper plate, with 3 cm of the end part of them sticking out of the device. To prevent errors, a number 18 screw was placed over the rods to tighten them (Fig. 2).

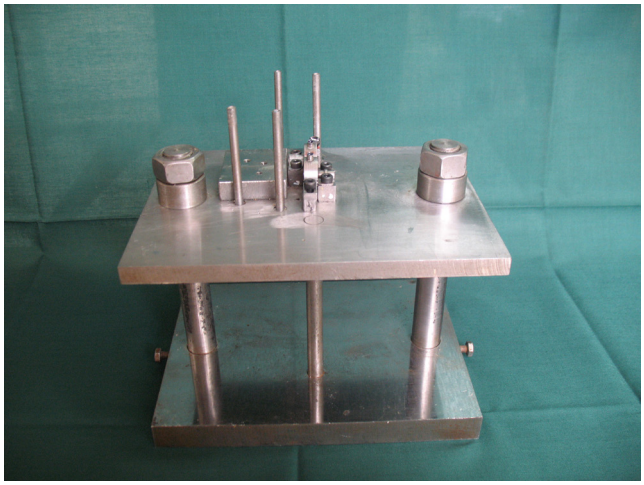

Fig. 1. Device used in the study

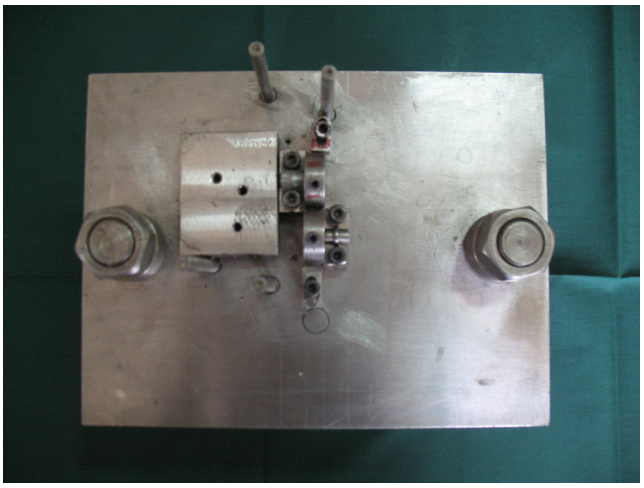

Fig. 2. Top view of the device

This device had 2 implant holders at the sites of teeth 29 and 31, which allowed changing the angulation of the implants. The implant holders were located on 2 spherical metal components measuring 22 mm in diameter and 8 mm in thickness. The location of an 8-millimeter-deep implant hole was determined using a surveyor with its axis directed at the center of the implant hole (Fig. 3). Shafts measuring 8 mm in larger diameter and 6 mm in smaller diameter were designed for the mobilization of the 2 described components. The assembly of the spherical metal component and the shaft was placed on a base measuring 25 mm in length, 10 mm in width and 11.5 mm in height. Two number 4 screws on each base attached them to the upper plate. For a better placement of the bases on the plate, 2 plates measuring 25 mm in length, 10 mm in width and 1 mm in depth were created and positioned lower than the level of the upper plate.

A plate measuring 50 mm in length, 40 mm in width and 12.3 mm in height was used to level the position of the master model and the implant holders. Four number 4 vertical rods were used around the master model on the device to standardize the path of insertion and retrieval of the tray (Fig. 4).

The implant holders were designed so that they could angulate each implant relative to the vertical axis (perpendicular to the occlusal plane and the device) by 0°, ±5°, ±10°, and ±15°. The center of rotation of both implant holders, which corresponded to the implant apex, was at the same level. To fix the desired angle, the position of both implant holders was secured with a metal blade. The 2 external hexagonal implants placed in the implant holders measured 4 mm in diameter and 15 mm in length (OSS415; Implant Innovations Inc., Palm Beach Gardens, USA). The impression of the master model was made using a prefabricated tray and an additional silicone impression material (A-Silicone; Zhermack, Badia Polesine, Italy), and the primary cast was poured. Two layers of base plate wax (TruWax®; Dentsply Trubyte, York, USA) were applied on the cast except for the stop points.[8,13] The cast was duplicated and turned into a stone model to standardize the space for the fabrication of trays for all samples. A light-cured acrylic resin (Preci-Tray; YETI Dental, Engen, Germany) was used for the fabrication of trays.

To fabricate a special tray for the open-tray impression technique, all of the aforementioned steps were repeated following the placement of the direct impression copings on the primary cast. Holes with a diameter of 3 mm, a 0.5-centimeter distance from each other and a wing were created on special trays. There were 2 holes at each side (a total of 4). The 4 vertical rods were passed through the 4 holes to determine the path of insertion of the tray. A metal plate weighing 430 g[16] was placed over the wing during the setting of the impression material to simulate hand pressure. The 2 implants could have 42 different positions relative to each other, with different angulation, including 6 angles for implant 5 and 7 angles for implant 7: 0°, ±5° and ±10° relative to the vertical axis for both implants; ±15° for the implant at the site of tooth 31 (implant 7), and –15° for the im-

Fig. 3. Accommodating the implant axe to the center of the implant hole by means of a surveyor

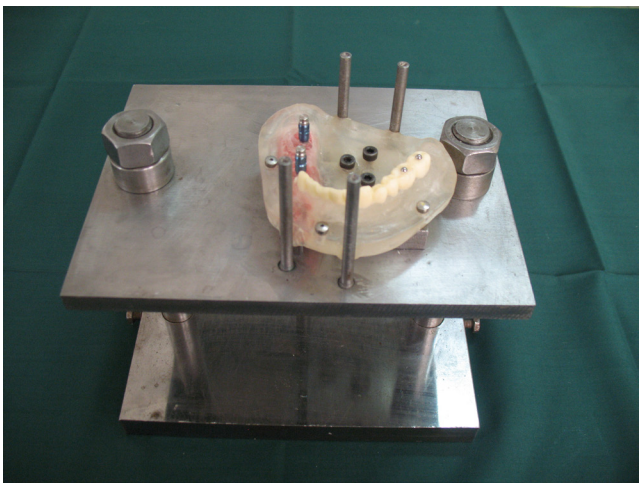

Fig. 4. Acrylic model on the device and the vertical guiding rods around it

plant at the site of tooth 29 (implant 5). Before making any impressions, the open and closed impression copings were attached to the implants of the master model. The basic three-dimensional (3D) measurements on the X, Y and Z axes were performed using a coordinate-measuring machine (CMM).

Before making the impression, a silicone adhesive material (Tray Adhesive; Zhermack) was applied on the internal margins and on 3 mm of the external margins of the fabricated tray, allowing 1 min to set, according to the manufacturer's instructions. A polyvinyl siloxane impression material (Monophase Medium Body, A-Silicone; Zhermack) was injected into the tray and around the impression copings (Fig. 5). The metal plate was placed over the wing, and the 4 vertical rods were passed through the 4 holes (Fig. 6). The tray stops matched the model stops and the tray was finally seated. After the final setting of the impression material, which took 5 min and 30 s, as stated in the manufacturer's instructions, the tray was removed from the model.

In the open-tray technique (direct pick-up coping), the direct impression copings were unscrewed prior to the removal of the impression and were separated from the model simultaneously with the removal of the impression (Fig. 7).

In the closed-tray technique (indirect transfer coping), the indirect impression copings were screwed into the implants, and after making the impression and separating them from the model, the copings were opened and attached to the implant analogs. The analog–coping complex was then placed in the impression.

Gingifast (Zhermack) was injected around the copings in the trays before pouring the casts. The metal balls were also placed at their respective locations in the impression. The final cast was poured with type IV dental stone (Zhermack) (Fig. 8). For the purpose of standardization, the powder to liquid ratio of the dental stone was determined according to the manufacturer's instructions, and mixing was performed with a vacuum mixer (Vac-U-Mixer; Whip Mix Corporation, Louisville, USA).

After the final setting of the gypsum (45 min), the final position of the 2 implants in the X, Y and Z axes relative to the reference points was determined using CMM with an accuracy of 0.1 μm (Fig. 9). A coordinate-measuring

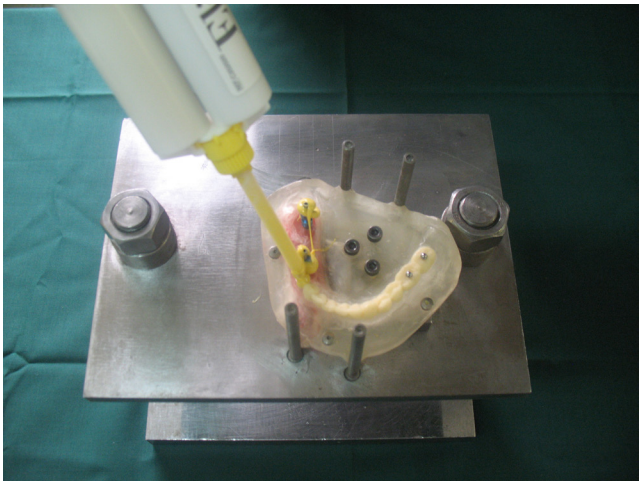

Fig. 5. Injecting the impression material over the copings

Fig. 6. Metal plate covering the wing during impression making

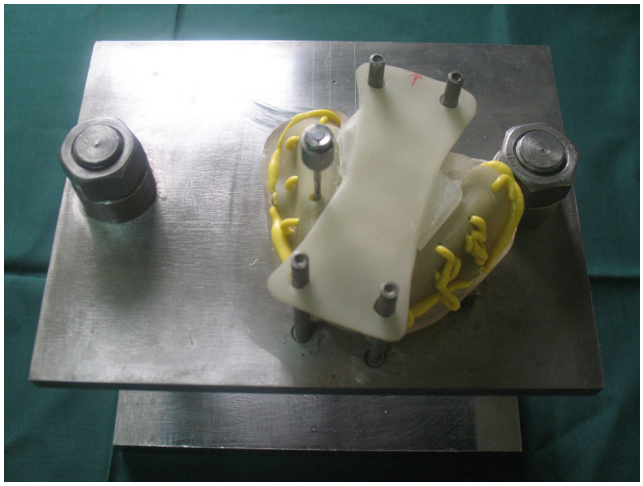

Fig. 7. Open-tray impression technique

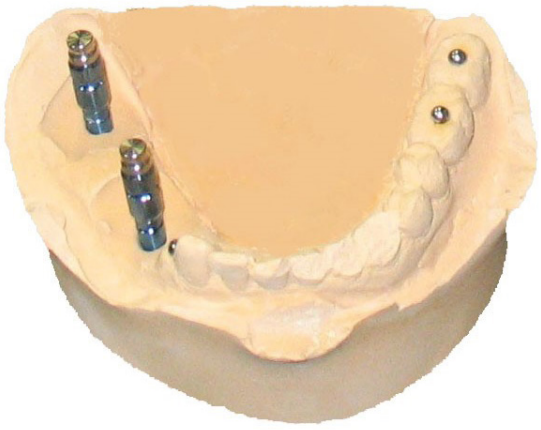

Fig. 8. Final cast

machine is a high-precision contact instrument with a 1 m × 1/8 m table and a sensitive probe that moves along the entire length and width of the table with 2 arms – vertical and horizontal. In a standard specific place on the table, there is a sphere with a fixed and reliable diameter so that the position of the probe and the tip size can be calibrated each time the probe is changed in terms of the 3 dimensions.

The sensitivity of the probe tip to contact is such that it measures the position of any point on the surface of an object relative to the standard sphere in the X, Y and Z dimensions with an accuracy of 0.1 μm over the entire area of the table. After providing settings and defining a spherical body by means of the Trimek software (Altube-Zuia, Spain), this device is able to specify the exact coordinates of the center of this sphere in relation to the standard sphere, and also indicate its out of sphericity (Fig. 10). In this study, the coordinates of the center of the direct and indirect impression copings relative to the 3 metal balls placed at the sites of teeth 18, 19 and 28 were determined in the 3 dimensions. The measurements were performed in triplicate, and the mean values were calculated and used for the statistical analysis.

Fig. 9. Coordinate-measuring machine (CMM)

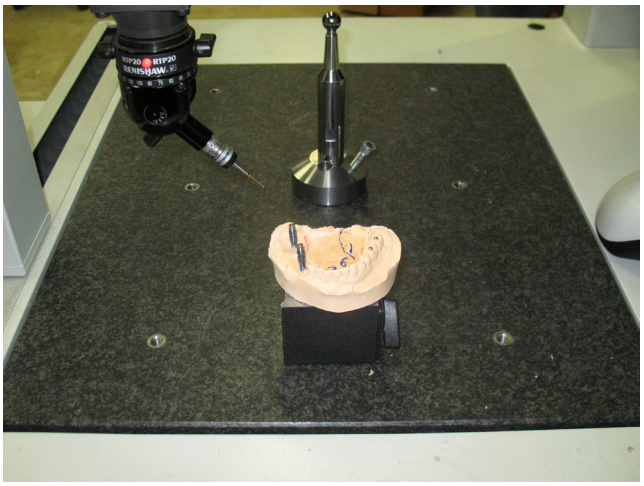

Fig. 10. Contact of the probe with the sphere

Statistical analysis

The coordinates of the center of each implant in the 3 dimensions in different impression techniques and at different angulation were determined, and differences with regard to the reference points were calculated.

The data was analyzed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). Differences in the X, Y and Z coordinates of the samples at different angulation, both in the open- and closed-tray techniques, were determined relative to the coordinates of the reference points, and the overall value in the 3 axes was calculated using the following formula (Equation 1):

$$\Delta R = \sqrt{(\Delta X)^2 + (\Delta Y)^2 + (\Delta Z)^2} \quad (1)$$

where:

R – spatial position;

X, Y, Z – coordinates in the 3 axes.

This was considered to be the dimensional accuracy of each impression. The statistical analysis was carried out using the independent *t* test and the one-way analysis of variance (ANOVA). Considering the significant effect of the angulation of the 2 implants relative to each other on the dimensional accuracy of the impressions, pairwise comparisons of different angulation were carried out using Tukey's test. A p-value <0.05 was considered statistically significant.

Results

The estimation of errors in the master cast in different implant positions revealed the maximum error in the parallel position of the 2 implants, when each of them was at an angle of –10° relative to the vertical axis (R = 1.3665). Also, the minimum error was noted when implant 5 was at an angle of –15° and implant 7 was at an angle of 10° relative to the vertical axis of the implant (R = 0.0188).

The impression error values in the parallel states of the 2 implants as well as in their different convergences and divergences according to their angular positions are presented in Table 1.

Table 1. Impression error values in the parallel, convergent and divergent positions of the 2 implants according to their angulation relative to the longitudinal axis

Position	Angulation relative to the longitudinal axis [°]		Number of positions	Impression error	*p*-value
	implant 5	implant 7			
Parallel	0	0	2	0.3104 ±0.04178	0.059
	5	5	2	0.7112 ±0.53412	
	10	10	2	0.0690 ±0.01858	
	–5	–5	2	0.1150 ±0.00000	
	–10	–10	2	1.3665 ±0.67123	
	–15	–15	2	0.8849 ±0.13626	
5° convergence	0	5	2	0.4360 ±0.00994	<0.001*
	–5	0	2	0.0662 ±0.02394	
	5	10	2	0.9317 ±0.16470	
	10	15	2	0.0739 ±0.01089	
	–10	–5	2	0.0229 ±0.01042	
	–15	–10	2	0.7555 ±0.05273	
10° convergence	0	10	2	0.2524 ±0.01513	<0.001*
	–10	0	2	0.0614 ±0.00740	
	5	15	2	1.2536 ±0.03511	
	–5	5	2	0.0514 ±0.02031	
	–15	–5	2	0.2557 ±0.00434	
15° convergence	0	15	2	0.5543 ±0.06516	0.578
	–15	0	2	0.2959 ±0.02914	
	–10	5	2	1.3048 ±1.78376	
	–5	10	2	0.0418 ±0.03786	
20° convergence	–15	5	2	0.0288 ±0.02071	0.005*
	–10	10	2	0.1081 ±0.02135	
	–5	15	2	0.2477 ±0.02627	
25° convergence	–15	10	2	0.0188 ±0.01102	0.086
	–10	15	2	0.1059 ±0.03732	
30° convergence	–15	15	2	0.6949 ±0.65102	–
5° divergence	0	–5	2	0.3914 ±0.06898	<0.001*
	5	0	2	0.9371 ±0.00040	
	10	5	2	0.0543 ±0.03306	
	–5	–10	2	0.7191 ±0.06766	
	–10	–15	2	0.9966 ±0.00399	
10° divergence	0	–10	2	0.2709 ±0.05986	<0.001*
	10	0	2	0.0660 ±0.01349	
	5	–5	2	0.9406 ±0.09869	
	–5	–15	2	0.9709 ±0.06278	
15° divergence	0	–15	2	0.7171 ±0.05593	0.014*
	5	–10	2	0.0940 ±0.09059	
	10	–5	2	0.0728 ±0.06300	
20° divergence	5	–15	2	0.0674 ±0.01360	0.002*
	10	–10	2	1.0299 ±0.05283	
25° divergence	10	–15	2	1.1336 ±0.06927	–

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

According to the results of ANOVA and the *t* test, a significant difference was observed in 5°, 10° and 20° convergences, and in 5°, 10°, 15°, and 20° divergences.

ANOVA showed that the parallel, convergent and divergent positions of the 2 implants relative to each other had no significant effect on the impression error ($p = 0.251$). The maximum mean impression error was noted in the 25° divergent position of the 2 implants (R = 1.1336) (Table 2).

The effect of the angulation of implants 5 and 7 relative to the vertical axis on the impression error was significant ($p < 0.0001$) (Table 3).

The results of the pairwise comparisons of different angulation with the use of Tukey's test did not show any significant differences in the 2 subgroups described below (implant 5 vs. implant 7):

- subgroup 1 (impression error range: 0.0188–0.3104): −15° vs. 10°; −10° vs. −5°; −15° vs. 5°; −5° vs. 10°; −5° vs. 5°; 10° vs. 5°; −10° vs. 0°; 10° vs. 0°; −5° vs. 0°; 5° vs. −15°; 10° vs. 10°; 10° vs. −5°; 10° vs. 15°; 5° vs. −10°; −10° vs. 15°; −10° vs. 10°; −5° vs. −5°; −5° vs. 15°; 0° vs. 10°; −15° vs. −5°; 0° vs. −10°; −15° vs. 0°; 0° vs. 0°; and
- subgroup 2 (impression error range: 0.3914–1.3665): 0° vs. −5°; 0° vs. 5°; 0° vs. 15°; −15° vs. 15°; 5° vs. 5°; 0° vs. −15°; −5° vs. −10°; −15° vs. −10°; −15° vs. −15°; 5° vs. 10°; 5° vs. 0°; 5° vs. −5°; −5° vs. −15°; −10° vs. −15°; 10° vs. −10°; 10° vs. −15°; 5° vs. 15°; −10° vs. 5°; −10° vs. −10°.

The impression technique (open- or closed-tray) had no significant effect on the impression error in different implant positions ($p = 0.390$). Accordingly, the mean error rate was 0.4460 ±0.4181 in the open-tray technique and 0.5245 ±0.5095 in the closed-tray technique. The difference in this respect between the 2 techniques was not significant ($p > 0.05$) (Table 4).

Table 2. Impression error values in the parallel, convergent and divergent positions of the 2 implants relative to each other

Position	Number of positions	Impression error	*p*-value
Parallel	12	0.5761 ±0.54887	0.251
5° convergence	12	0.3810 ±0.37747	
10° convergence	10	0.3749 ±0.47262	
15° convergence	8	0.5492 ±0.84289	
20° convergence	6	0.1282 ±0.10072	
25° convergence	4	0.0624 ±0.05505	
30° convergence	2	0.6949 ±0.65102	
5° divergence	10	0.6197 ±0.37407	
10° divergence	8	0.5621 ±0.43095	
15° divergence	6	0.2946 ±0.33203	
20° divergence	4	0.5486 ±0.55655	
25° divergence	2	1.1336 ±0.06927	

Data presented as *M* ±*SD*.

Table 3. Impression error values in different angular positions of implants 5 and 7 relative to the vertical axis of the implant (ANOVA)

Angulation [°]		Impression error	*p*-value
implant 5	implant 7		
0	0	0.3104 ±0.04178	0.0001*
5	5	0.7112 ±0.53412	
10	10	0.0690 ±0.01858	
−5	−5	0.1150 ±0.00000	
−10	−10	1.3665 ±0.67123	
−15	−15	0.8849 ±0.13626	
0	5	0.4360 ±0.00994	
0	10	0.2524 ±0.01513	
0	15	0.5543 ±0.06516	
0	−5	0.3914 ±0.06898	
0	−10	0.2709 ±0.05986	
0	−15	0.7171 ±0.05593	
5	0	0.9371 ±0.00040	
10	0	0.0660 ±0.01349	
−5	0	0.0662 ±0.02394	
−10	0	0.0614 ±0.00740	
−15	0	0.2959 ±0.02914	
5	10	0.9317 ±0.16470	
5	15	1.2536 ±0.03511	
5	−5	0.9406 ±0.09869	
5	−10	0.0940 ±0.09059	
5	−15	0.0674 ±0.01360	
10	5	0.0543 ±0.03306	
−5	5	0.0514 ±0.02031	
−10	5	1.3048 ±1.78376	
−15	5	0.0288 ±0.02071	
10	15	0.0739 ±0.01089	
10	−5	0.0728 ±0.06300	
10	−10	1.0299 ±0.05283	
10	−15	1.1336 ±0.06927	
−5	10	0.0418 ±0.03786	
−10	10	0.1081 ±0.02135	
−15	10	0.0188 ±0.01102	
−5	15	0.2477 ±0.02627	
−10	15	0.1059 ±0.03732	
−15	15	0.6949 ±0.65102	
−5	−15	0.9709 ±0.06278	
−10	−5	0.0229 ±0.01042	
−15	−5	0.2557 ±0.00434	
−5	−10	0.7191 ±0.06766	
−10	−15	0.9966 ±0.00399	
−15	−10	0.7555 ±0.05273	

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

Table 4. Mean impression error values in the open- and closed-tray techniques

Angulation [°]		Open-tray technique	Closed-tray technique
implant 5	implant 7		
0	0	0.2808	0.3399
5	5	0.3335	1.0889
10	10	0.0558	0.0821
–5	–5	0.1150	0.1150
–10	–10	1.8411	0.8918
–15	–15	0.9812	0.7885
0	5	0.4431	0.4290
0	10	0.2417	0.2631
0	15	0.5082	0.6004
0	–5	0.3427	0.4402
0	–10	0.2286	0.3132
0	–15	0.6776	0.7567
5	0	0.9374	0.9369
10	0	0.0756	0.0565
–5	0	0.0831	0.0492
–10	0	0.0667	0.0562
–15	0	0.2753	0.3166
5	10	1.0482	0.8152
5	15	1.2287	1.2784
5	–5	1.0104	0.8709
5	–10	0.0229	0.1581
5	–15	0.0770	0.0578
10	5	0.0777	0.0310
–5	5	0.0657	0.0370
–10	5	0.0435	2.5661
–15	5	0.0434	0.0141
10	15	0.0662	0.0816
10	–5	0.0282	0.1173
10	–10	0.9925	1.0672
10	–15	1.0846	1.1826
–5	10	0.0151	0.0686
–10	10	0.1231	0.0930
–15	10	0.0111	0.0266
–5	15	0.2291	0.2663
–10	15	0.0795	0.1323
–15	15	0.2346	1.1552
–5	–15	0.9265	1.0153
–10	–5	0.0155	0.0302
–15	–5	0.2587	0.2526
–5	–10	0.6713	0.7670
–10	–15	0.9938	0.9995
–15	–10	0.7182	0.7928
Total		0.4181	0.5095

Discussion

Several methods are employed to achieve passive fit in implant-supported restorations. An accurate impression without distortion is imperative to precisely reproduce the position of implants on casts. Considering the problems encountered when making the impressions of angulated implants, this study assessed the effect of dental implant angulation on the dimensional accuracy of master casts and showed that the angulation of the 2 implants (5 and 7) relative to their longitudinal axis in different positions had a significant effect on the dimensional accuracy of the final casts. Conversely, the effect of the impression technique (open- or closed-tray) in the parallel, convergent and divergent positions of the implants on the impression error was not significant. In other words, implant angulation affected the impression error.

Errors in impression making may be related to the type of impression material, the technique of impression making, the performance of the dentist, the incorrect position of impression copings, and the incorrect attachment of implant components. Although the passive fit of implants has been the topic of many investigations, the clinical significance of dimensional changes or distortion has not been adequately emphasized. Also, the acceptable range of errors in the transfer of the intraoral implant position to the dental cast has not been determined yet. The findings of this study were related to the placement of implants 5 and 7 in 42 different positions at the –15°, –10°, –5°, 0°, 5°, 10°, and 15° angles relative to their longitudinal axis, and may not be generalizable to a higher number or other angulation of implants. However, it seems that other angulation has limited application in the clinical setting.

In this study, no specific ascending or descending patterns were noted regarding the impression error in the different angulation of the 2 implants to obtain a definite conclusion in this respect. However, in total, the effect of implant angulation on the impression error was significant (p = 0.0001). The significant effect of implant angulation on the dimensional accuracy of the final casts highlights the importance of this topic, especially because implants are placed in angulated positions in many patients. Despite the significance of this topic, studies addressing this issue are limited. Carr[17] and Assuncao et al.[18] reported that angulated implants had lower dimensional accuracy than straight implants, while another study evaluating 3 implants determined that the implant angle had no significant effect on the dimensional accuracy of casts.[19] It seems that when a higher number of implants with different angulation is used, the dimensional changes of the impression material increase.[4] Conrad et al. evaluated the accuracy of impression techniques when 3 angulated implants were placed at 3 corners of a triangle at angles of 5°, 10° and 15° (convergent or divergent relative to the central implant).[4] They dem-

onstrated that the implant angle had a significant effect on the dimensional accuracy of casts.[4] Their findings are in line with the present study, although we used 2 implants and evaluated the 0° angle as well. Carr compared the dimensional accuracy of the final casts obtained with 2 impression techniques for 2 divergent implants (15°).[17] The author showed that this divergence had no significant effect on the dimensional accuracy of impressions. However, straight implants had slightly higher dimensional accuracy than angulated implants. This finding was attributed to the proximity of implant locations (11 mm). In contrast to the present study, the author evaluated only one implant angle.[17] Choi et al. assessed the accuracy of 2 impression techniques for parallel or 8° divergent internal hexagonal implants, and showed that the accuracy of implant impressions was the same in both impression techniques and a degree of divergence up to 8° had no significant effect on the accuracy of impressions.[19] The non-significant effect of the degree of convergence/divergence on the dimensional accuracy of impressions was confirmed in this study.

This study found no significant difference in the dimensional accuracy of the casts when the open- and closed-tray impression techniques were compared. A review of the literature revealed that in the presence of 4 or more implants, the open-tray technique often provides higher accuracy than the closed-tray technique.[20] However, studies on 3 or fewer implants mainly found no significant difference between the impression techniques in terms of dimensional accuracy.[7,10,17] The results of the current study confirm this finding. Since some degrees of error and deformity are inevitable in both techniques, attempts must be made to minimize distortion during different phases of impression making as well as during the transfer of the implant position to the final cast in order to fabricate stress-free implant-supported restorations.[21,22] Carr found no significant difference between the open- and closed-tray impression techniques when making the impressions of 2 implants with 15° divergence.[17] Conrad et al. evaluated 3 implants and reported a similar level of distortion and deformity for the 2 impression techniques.[4] Herbst et al. found no significant difference in dimensional accuracy between different impression techniques.[7]

Similar to previous research, this study showed that the distances measured on the original model could not be ideally transferred to the final cast, and that the spatial coordinates of the implants changed relative to each other when transferred to the cast.[10,23,24] The reason might be the movement of the metal copings when opening or closing the guiding rods or attaching the analog,[10,25] the dimensional changes of the gypsum,[26] the shrinkage of the acrylic resin when the copings were connected to each other,[10,25,26] changes in the impression material,[25] the depth of implant placement,[27] soft tissue adhesion (since it could modify the mucosal aspect around the implant),[28] the duration of the use of the dental stone,[29] the implant–abutment interface,[30] machining tolerance (0.6–136 μm),[20] or the operator's error, which is minimally 30 μm in the laboratory setting, but may increase in the oral environment.[31] Moreover, different methodologies can yield variable results. The designed experimental models (metal or acrylic), different measuring devices, the distances measured relative to the reference points, and different methods of attachment of metal copings can all affect the results. Also, some studies measured the distances in only the X and Y dimensions, while others, including the current study, measured dimensional changes in all 3 dimensions of X, Y and Z, and calculated the spatial position (R), which would enhance the comparison of the results of studies.

There are limitations to this study. It evaluated only 2 implants in a limited range of angulation. Also, the effect of the type of impression material on the dimensional accuracy of casts was not evaluated. Future studies with a higher number of implants, a larger range of angulation (>15°) and different impression materials are required to better elucidate this topic and find a definite conclusion, generalizable to the clinical setting while controlling for a higher number of confounders.

Conclusions

Within the limitations of this in vitro study, the results showed a significant effect of implant angulation (implants 5 and 7) in the range from –15° to +15° on the dimensional accuracy of impressions. The open- and closed-tray techniques were not significantly different in this respect. Under similar conditions, it seems that the closed-tray technique may be preferred due to the ease of use, patient comfort and accuracy comparable to that obtained with the open-tray technique.

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Effect of the local application of bupivacaine in early pain control following impacted mandibular third molar surgery: A randomized controlled study

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Abstract

Background. Postoperative pain is one of the main complications following impacted mandibular third molar (IMTM) surgery.

Objectives. The aim of this study was to assess the effect of the local application of bupivacaine on reducing early postoperative pain following IMTM surgery.

Material and methods. A prospective, single-blinded, randomized controlled study was conducted on 40 patients who had undergone the surgical removal of an IMTM under local anesthesia. In the study group ($n = 20$), absorbable gelatin sponge (AGS) soaked in 3 mL of 0.5% plain bupivacaine hydrochloride was locally applied in the post-extraction socket. In the control group ($n = 20$), AGS soaked in 3 mL of normal saline was used. Pain intensity was assessed using a pain numerical rating scale (NRS) 4 and 12 h postoperatively. The variables were compared between the 2 groups and probability values <0.05 were considered statistically significant.

Results. The pain scores in the study group were significantly lower than those recorded in the control group at 4 h postoperatively ($p = 0.003$), whereas the difference in the pain scores between the 2 groups 12 h after surgery was not statistically significant ($p = 0.443$).

Conclusions. The local application of bupivacaine is effective in reducing postoperative pain 4 h after the surgical extraction of IMTMs without any significant complications.

Keywords: third, postoperative, pain, bupivacaine, molar

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Introduction

The impaction of third molars is a common problem worldwide. Its prevalence has been estimated to range from about 3% to about 68%, with an average rate of 24.4%; it has significant geographic variation.[1] The surgical extraction of impacted mandibular third molars (IMTMs) is a common practice in oral surgery and it is usually accompanied by side effects, such as swelling, trismus and postoperative pain.[2–5]

Postoperative pain is the most common complication following dental surgery,[3] and the patient's experience depends on the degree of surgical trauma, the need for bone removal and the periosteal extension. It is undoubtedly the most common symptom and can continue even 1 week after surgery, despite analgesic treatment, leading to increased morbidity and detrimental effects on the patient's well-being and quality of life.[6,7] Inadequate management of early postoperative pain has been related to a wide range of negative outcomes, including delayed recovery and even an increased risk of developing chronic pain.[8,9]

The development and use of local anesthetics for intraoperative anesthesia and postoperative analgesia is considered to be one of the most important advances in dentistry in the last century.[10] Several researchers have credited bupivacaine with the ability to provide prolonged postoperative analgesia and to minimize analgesic requirements in the early postoperative hours, when pain is at its highest intensity. Its long duration of action and superior ability to decrease pain and discomfort have been reported in comparison with articaine and lidocaine. Therefore, bupivacaine is a common choice for pain control following IMTM extraction.[11]

The delivery of a local anesthetic agent to the post-extraction socket has been described to alleviate postoperative pain.[3] However, the use of local delivery therapeutics requires some mechanism to maintain the agent within the oral environment for an adequate period of time to prolong drug effectiveness and reduce its toxicity by slowing drug uptake into the systemic circulation.[12,13]

Few studies have investigated the effect of the local application of bupivacaine in post-extraction sockets following the surgical removal of IMTMs. Therefore, the aim of this study was to assess the effect of the local application of bupivacaine on reducing early postoperative pain following IMTM surgery.

Material and methods

This study was designed and conducted as a prospective, single-blinded, randomized controlled study guided by the Consolidated Standards of Reporting Trials (CONSORT) statement.[14] It included patients undergoing the surgical extraction of IMTMs at the Department of Oral and Maxillofacial Surgery of the College of Dentistry, University of Baghdad, Iraq.

The inclusion criteria were as follows: adults aged 18 years or older; with the American Society of Anesthesiologists physical status classification level I (ASA I); who had an IMTM indicated for surgical extraction; and reported no history of allergic reactions to the local anesthetic agents used in the study.

The exclusion criteria embraced patients with systemic diseases, pregnant females, patients with a missing lower second molar on the side of the surgery, patients with impacted teeth due to any pathology, such as cysts or tumors, patients presenting with signs and symptoms of acute general infections, such as fever and lymphadenopathy, and patients who had taken any type of analgesic within 24 h prior to the surgery.

The study was approved by the institutional Research Ethics Committee (protocol No. 127119). The patients were informed about the nature of the study, the procedures, and the possible intra- and postoperative complications they might encounter. Every patient signed an informed consent form to participate in the study.

The patients were randomly assigned to 2 groups with the use of Microsoft Office Excel 2016 (Microsoft Corporation, Redmond, USA). Block randomization was performed to ensure a nearly equal distribution of patients in both groups. For the patients in the study group, a 10 × 10 × 10-mm piece of absorbable gelatin sponge (AGS) (SPONGOSTAN® Dental; Ferrosan Medical Devices, Søborg, Denmark) soaked in 0.5% plain bupivacaine hydrochloride (Marcaine; AstraZeneca, Istanbul, Turkey) was applied in the post-extraction socket. The patients assigned to the control group received AGS soaked in normal saline as a placebo. The patients were blinded to the type of local treatment they received.

A preoperative panoramic radiograph was obtained for each patient to assess the angulation of the impacted tooth according to Winter's classification[15] as well as the position and depth of impaction according to the Pell and Gregory classification.[16] All procedures were scheduled to start at 9 a.m. and were performed by one operator under local anesthesia (2% lidocaine hydrochloride with epinephrine 1:80,000). The procedure consisted of reflecting a triangular flap and extracting the tooth with the use of elevators alone, or after bone removal with or without tooth sectioning with the use of a surgical handpiece and burs under copious irrigation with normal saline. The duration of the surgery was calculated in minutes starting from the first incision to the last suture.

Operative difficulty was determined by the surgical technique and the duration of extraction according to de Carvalho and Vasconcelos.[17] With respect to the surgical technique, the degree of difficulty was considered low when the extraction was performed using elevators alone, moderate when bone removal was required, and high when bone removal and tooth sectioning were required to complete the extraction. For the duration of surgery, the difficulty was considered low when the duration

of the surgical extraction was less than 15 min, moderate when the duration was 15–30 min and high when the duration was more than 30 min.

After extraction, the socket was thoroughly irrigated with normal saline, all sharp edges were smoothened and hemostasis was achieved. In the study group, a piece of AGS was soaked in 3 mL of 0.5% plain bupivacaine hydrochloride for 5 min and applied in the post-extraction socket. In the control group, the same procedure was performed, but 3 mL of normal saline was used. The flap was repositioned to its original place and sutured with 3/0 black silk sutures, using the simple interrupted suturing technique. Antibiotics (amoxicillin 500 mg every 8 h for 5 days) and ibuprofen (400 mg every 8 h for 5 days) were prescribed to all patients, and the patients were instructed to record any extra analgesic drug use.

Pain intensity was reported by the patients on a pain numerical rating scale (NRS) 4 and 12 h postoperatively. The patients were instructed to write a number (0–10) to quantify their pain intensity, where 0 represented no pain and 10 represented the worst pain possible. The patients were aware that the scale served to analyze the presence and intensity of pain alone, and was not a representation of generalized postoperative discomfort.

The predictor variable was the local application of bupivacaine- or normal saline-soaked AGS in the post-extraction socket, while the outcome variable was the pain scores recorded 4 and 12 h after surgery. Other variables included age, gender, indication for extraction, the angulation, position and depth of the IMTM, and the difficulty of extraction determined by the surgical technique and the duration of extraction.

Statistical analysis

The statistical analysis was performed using GraphPad Prism v. 6 for Windows (GraphPad Software, La Jolla, USA). The descriptive statistical analysis included the calculation of frequency as number (*n*), mean (*M*) and standard deviation (*SD*), and median (*Me*). The inferential analysis included the Shapiro–Wilk normality test, the Mann–Whitney *U* test, Cohen's *d* test, Fisher's exact test, and the χ^2 test. Probability values <0.05 were considered statistically significant.

Results

Forty-three patients initially participated in this study, but 3 patients were lost to follow-up and excluded from the analysis. The remaining 40 patients ranged in age from 18 to 30 years, with a mean age of 23.6 ±3.55 years. Among the patients there were 17 (42.5%) males and 23 (57.5%) females. They were randomly assigned to the control and study groups of 20 patients each. Figure 1 summarizes the phases of the study.

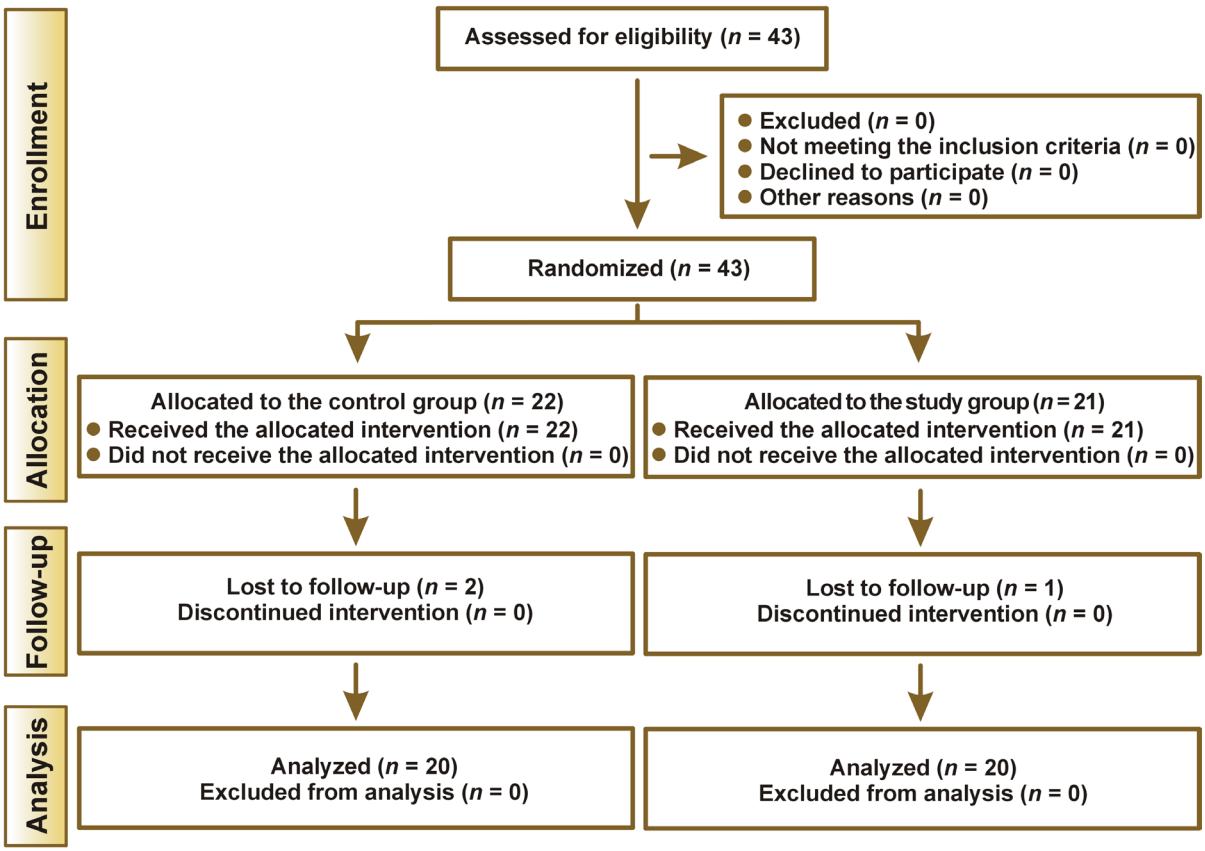

Fig. 1. Flow diagram of the randomized study

The comparisons of different independent variables between the 2 groups are summarized in Table 1. All differences were statistically non-significant, which indicates that none of these variables acted as a confounding factor for the outcome of interest (the pain score).

Four hours postoperatively, the study group recorded a mean pain score of 2.90 ±2.43 and a median of 3.0, while the control group recorded a mean pain score of 5.95 ±3.14 and a median of 5.5. The difference in the pain scores between the 2 groups was statistically significant (p = 0.003) (Fig. 2). The effect size of the intervention was 0.9723 (Glass's delta), which can be interpreted as a large effect (large effect: 0.8 or more).

Twelve hours after surgery, the pain scores recorded by the study group presented a mean of 3.80 ±3.09 and a median of 3.0, while the control group had a mean of 4.25 ±2.71 and a median of 3.0. The difference between the 2 groups for this time interval was not statistically significant (p = 0.443) (Fig. 2).

None of the patients in either group required extra analgesics other than those prescribed during the first 24 h postoperatively. The sutures were removed 7 days postoperatively. Five patients in the control group developed complications (a dry socket in 2 patients and wound dehiscence in 3 patients). Three patients in the study group developed complications (a dry socket in 2 patients and wound dehiscence in 1 patient). The difference in complication rates between the 2 groups was not statistically significant (p = 0.695).

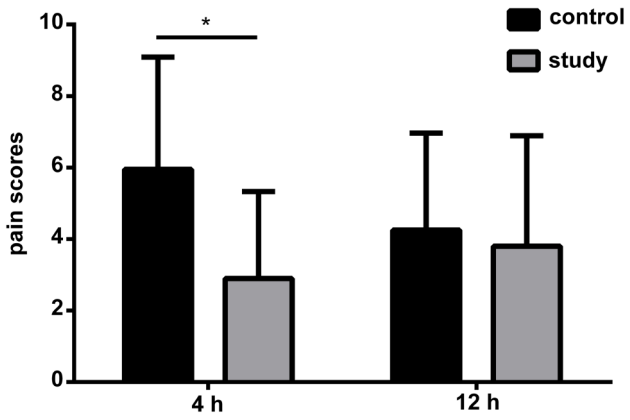

Fig. 2. Bar chart illustrating the difference in the pain scores between the 2 groups 4 and 12 h postoperatively

* statistically significant.

Table 1. Comparison of independent variables between the 2 groups

Variable		Control group n = 20	Study group n = 20	p-value
Age [years] $M \pm SD$		24.8 ±3.61	23.4 ±3.57	0.6619†
Gender n	male	10	7	0.5231††
	female	10	13	
Side of extraction n	right	9	13	0.3406††
	left	11	7	
Angulation (Winter's classification) n	mesioangular	8	6	0.2502‡
	vertical	7	4	
	horizontal	5	10	
Position (Pell and Gregory classification) n	A	7	9	0.7475††
	B	13	11	
Indication for extraction n	dental caries	3	6	0.5177‡
	orthodontic treatment	9	7	
	pericoronitis	8	7	
Duration of extraction [min] $M \pm SD$		27.55 ±12.21	30.55 ±14.18	0.6054†
Difficulty/duration n	low	1	0	0.5378‡
	moderate	10	12	
	high	9	8	
Difficulty/technique n	low	4	4	0.7788‡
	moderate	9	7	
	high	7	9	

n – number; M – mean; SD – standard deviation; † Mann–Whitney U test; †† Fisher's exact test; ‡ χ^2 test.
All patients demonstrated class II according to the Pell and Gregory classification.

Discussion

This study evaluated the efficacy of AGS soaked in plain bupivacaine or normal saline, applied in the post-extraction socket, in reducing the early postoperative pain associated with IMTM surgery. This approach is in line with Shepherd et al., who maintained that the treatment of postoperative pain following dental extraction requires a formulation that would simultaneously serve as a hemostatic agent as well as a vehicle for controlled anesthetic delivery.[13] The authors tested a biodegradable dental material containing bupivacaine combined with an absorbable hemostat Gelfoam® in a rat animal model, where it was packed in post-extraction sockets, and they observed reduced post-extraction pain by assessing food consumption, body weight, and sensitivity to mechanical and thermal stimuli.[13]

The present study indicates that the local application of 0.5% bupivacaine hydrochloride in the manner described above provided effective pain relief in the first 4 h after IMTM surgery. Studies have shown that the maximum postoperative dental pain after the surgical extraction of IMTMs is usually experienced during the first 6–12 h,[3,18] so the use of bupivacaine in this simple-to-apply method can be helpful for patients in the early postoperative period.

Khiavi et al. used 4 mL of 0.5% plain bupivacaine hydrochloride to irrigate the post-extraction sockets of IMTM patients once, before flap suturing, and compared it with normal saline irrigation in a randomized clinical study with a crossover design.[3] Pain was assessed at 4 time intervals – 1, 6, 12, and 24 h postoperatively. The authors reported significant pain alleviation at all time intervals, with no complications.[3]

In their study, Talimkhani et al. used the intra-socket administration of bupivacaine with a prepared catheter fixated to a mandibular second molar, with its cannula inserted into the socket of the extracted third molar so that the patients could directly administer drugs (bupivacaine or saline as a placebo) into the tooth socket.[19] They compared it with oral mefenamic acid capsules or capsules filled with milk powder (as a placebo) in a randomized clinical study. The patients recorded the perceived pain intensity on a visual analogue scale (VAS) every 2 h before and after drug administration for 24 h. The authors found that bupivacaine significantly relieved postoperative pain after the surgical extraction of IMTMs.[19]

To assess the clinical effectiveness of the local application of bupivacaine in relieving early post-extraction pain, the amount of improvement that is important to patients must be determined. It is not enough to consider only the statistical significance, but also whether the observed change is meaningful to patients.[20] The distribution-based method was used in the present study to determine whether the pain relief achieved with the local application of bupivacaine 4 h after surgery was more than would be expected by chance alone, and it demonstrated that the local application of bupivacaine, as described in the present study, had a considerable clinical effect.

The difference in the pain intensity recorded 12 h after surgery was not statistically significant, which indicates that this method of local application of bupivacaine provided only temporary relief of postoperative pain. In the present study, postoperative pain during the first 24 h was assessed at 2 time intervals only (4 and 12 h after surgery) to ensure better patient compliance. Talimkhani et al. reported more prolonged pain relief at 6 time points with the use of their method of multiple intra-socket administration, which required more patient compliance,[19] while Khiavi et al. reported pain relief that lasted for 24 h from a single irrigation of the post-extraction socket at the end of surgery.[3]

No major complications were encountered in this study and the difference between the 2 groups in this regard was not statistically significant, indicating that this method of local application of bupivacaine is safe to use.

Limitations

The main limitations of this study are its small sample size and the fact that the sample size estimation was not performed. However, the total number of patients in this study (40) is close to that reported in 2 other studies that investigated the local application of bupivacaine after IMTM extraction.[3,19] Other limitations of the study are the fact that the operator was not blinded to the intervention and the fact that the assessment of postoperative pain during the first 24 h was performed at only 2 time intervals to ensure better patient compliance.

Conclusions

The local application of AGS soaked in plain bupivacaine hydrochloride significantly reduces postoperative pain 4 h following IMTM surgery without significant complications.

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Effects of non-surgical periodontal therapy on serum inflammatory factor high-sensitive C-reactive protein, periodontal parameters and renal biomarkers in patients with chronic periodontitis and chronic kidney disease

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Abstract

Background. Chronic kidney disease (CKD) is associated with significant morbidity and mortality, and there are various risk factors for this disease. Although the association between CKD and periodontal disease (PD) has been reported in various cross-sectional studies, longitudinal intervention studies are scarce.

Objectives. This study aimed to evaluate the effects of non-surgical periodontal therapy (NSPT) on periodontal clinical parameters, serum inflammatory factor high-sensitivity C-reactive protein (hs-CRP) and renal biomarkers in patients with CKD and chronic periodontitis (CP).

Material and methods. A total of 80 patients with confirmed CKD aged 22–65 years, attending the Institute of Kidney Diseases Research Centre (IKDRC) in Ahmedabad, India, and referred to the Government Dental College and Hospital, Ahmedabad (GDCHA), were enrolled in this study. The patients were divided into 2 groups: group 1 received NSPT, including scaling and root planing (SRP), as well as oral hygiene instructions; and group 2 received oral hygiene instructions without NSPT. Periodontal clinical parameters, such as probing pocket depth (PPD), clinical attachment loss (CAL), bleeding on probing (BoP), the periodontal inflamed surface area (PISA) score, and the Simplified Oral Hygiene Index (OHI-S), were recorded. Biomarkers, including hs-CRP, the estimated glomerular filtration rate (eGFR) and the urine albumin-to-creatinine ratio (UACR), were obtained from medical records. The comparisons of periodontal parameters, hs-CRP and renal biomarkers within and between the groups were performed at baseline, and 3 and 6 months after treatment.

Results. The periodontal parameter scores as well as the serum levels of hs-CRP and UACR significantly decreased while eGFR significantly increased in group 1 after treatment as compared to baseline ($p < 0.001$). Six months after treatment, group 1 showed significantly lower values than group 2 for periodontal parameters, the serum levels of hs-CRP and renal biomarkers except for eGFR, which improved and increased ($p < 0.001$).

Conclusions. Periodontitis is an important source of chronic inflammation and the treatment of periodontitis can hinder systemic inflammation in CKD patients. Non-surgical periodontal therapy resulted in improved periodontal health, with significant decreases in hs-CRP and UACR, and an increase in eGFR in CKD patients with CP in comparison with CKD patients not receiving NSPT.

Keywords: chronic renal insufficiency, glomerular filtration rate, periodontal disease, urine albumin-to-creatinine ratio

Introduction

There is a well-known saying that "oral health is equal to overall health". The oral cavity is the intersection of dentistry and medicine, semi-independent fields that share the common goal of improving the health and quality of life of patients. Since the 1980s, research has considered the possibility that poor oral health substantially affects overall health.[1] Chronic periodontitis (CP) is the most common oral inflammatory condition studied in this context. According to the 2010 Global Burden of Disease (GBD) Study, CP is the 6th most prevalent condition, affecting 10.8% (95% uncertainty interval (*UI*): 10.1–11.6), i.e., 743 million people aged 15–99 worldwide.[2] Chronic periodontitis is caused by dysbiotic oral biofilm that destroys the supporting connective tissues; a cascade of inflammatory immune responses fails to resolve the dysfunction and the dysregulated chronic inflammation ensues in a susceptible host. This results in the formation of periodontal pockets with chronically ulcerated pocket epithelium.[3] In severe disease cases, the surface area of the ulcerated epithelium can be as large as 40 cm^2, which increases microbial tissue invasion and perpetuates systemic inflammation accompanied by a rise in inflammatory biomarkers in serum, such as C-reactive protein (CRP) and interleukin (IL)-6.[4]

The course of non-communicable diseases, like atherosclerosis, myocardial infarction, non-hemorrhagic cerebrovascular disease, and diabetes mellitus, or adverse pregnancy outcomes can be related to the progression and treatment of periodontal disease (PD).[5] This concept has been expanded to include systemic diseases, such as rheumatoid arthritis, malignant neoplasia and chronic kidney disease (CKD).[5] Chronic kidney disease remains a prevalent public health problem. In 2017, the global prevalence of CKD was estimated at 9.1% (697.5 million cases), with over a third of CKD patients living in 2 countries, namely China (132.3 million) and India (115.1 million).[6] Chronic kidney disease is defined as either the occurrence of kidney damage or a decreased kidney function, wherein the estimated glomerular filtration rate (eGFR) amounts to <60 mL/min/1.73 m^2 for 3 months or longer.[7] Chronic kidney disease is largely preventable and treatable. The trend of an increasing incidence of CKD as a direct cause of global morbidity and mortality, and also as an important risk factor for cardiovascular diseases (CVD) poses major challenges for both healthcare and the economy in the near future.

The association between CP and CKD has been studied widely since the 2000s and CP is now considered a novel, non-traditional risk factor for CKD.[8,9] Chronic periodontitis may have its share in CVD-related mortality in CKD patients.[9] However, prospective and interventional studies on this association with long follow-up periods are scarce. Thus, the present study was conducted to address this research gap by evaluating the effects of non-surgical periodontal therapy (NSPT) on clinical parameters, serum inflammatory factor high-sensitivity C-reactive protein (hs-CRP) and renal biomarkers in patients with CKD and CP.

Material and methods

A randomized, controlled, parallel-group, single-center pilot study was conducted from July 2018 to June 2019.

Ethical approval and consent

Before the commencement of this study, the research protocol was submitted to the institutional ethics committee at Government Dental College and Hospital, Ahmedabad (GDCHA), India (IEC GDCH/S.6/2018, dated March 15, 2018), and subsequently approved. This study was registered in the Clinical Trials Registry – India (CTRI trial No. REF/2019/09/028096, dated September 14, 2019). The study was conducted in accordance with the 2020 Declaration of Helsinki and written informed consent was obtained from all patients.

Participants and eligibility criteria

Patients suffering from CKD, attending the outpatient department of the Institute of Kidney Diseases and Research Centre (IKDRC) in Ahmedabad, India, and referred to the Department of Periodontology of GDCHA were enrolled in this study. The inclusion criteria were as follows: male or female patients aged 18 years or older; patients with high-risk CKD, already receiving treatment for CKD at IKDRC, with eGFR < 60 mL/min/1.73 m^2 for 3 months or longer, and the urine albumin-to-creatinine ratio (UACR) ≥70 mg/gCr on 3 consecutive occasions; patients having generalized moderate-to-severe periodontitis, i.e., with a minimum cumulative probing pocket depth (PPD) of 40 mm (defined as the sum of the greatest PPDs measured for each tooth, excluding PPDs < 5 mm); and patients who provided informed consent to participate. The exclusion criteria were as follows: patients suffering from end-stage renal disease (ESRD) and requiring treatment with renal replacement therapy (RRT); patients receiving immunosuppressive medications; patients with a history of infective endocarditis or a heart valve replaced/repaired with prosthetic material; patients who underwent any periodontal treatment in the previous year; and patients who could not be subjected to periodontal treatment, e.g., with severe bleeding disorders that contraindicate periodontal treatment.

Randomization and allocation

After considering the eligibility criteria, a total of 100 patients were enrolled. Randomization was based on a simple randomization method, wherein the patients enrolled on days 1–15 of the month were assigned to group 1 (test group) and those enrolled on days 16–30/31 of the month (16–28 for February 2019) were assigned to group 2 (control group) (Fig. 1).

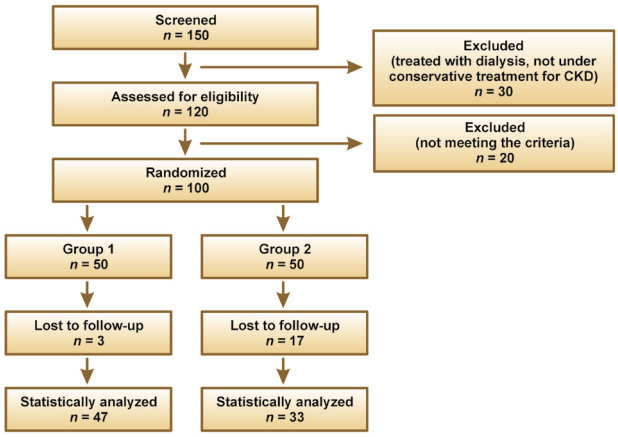

Fig. 1. Flowchart of the study

Group 1 patients received intensive NSPT, including supra/subgingival scaling and root planing (SRP) under local anesthesia, as well as oral hygiene instructions. Teeth with a poor prognosis and PPD > 8 mm, grade III mobility in lateral and vertical directions, and/or retained infected root pieces were extracted during the baseline visit. The patients were supported with a maintenance program for a period of 6 months. Oral hygiene instructions were reinforced at each appointment. The patients were provided with a manual toothbrush and fluoridated toothpaste for maintaining good oral hygiene.

Group 2 patients received only oral hygiene instructions without any NSPT. The patients were monitored for the following 6 months at 3-month intervals.

All interventions were provided by the principal investigator, who was blinded to the group allocation. The patients were followed up to assess the effects of the interventions at 3 and 6 months after the intervention.

Clinical examination and data collection

The data was collected with regard to 3 sections. Section 1: Demographic details, including age, gender, the socio-economic status (SES) assessed by the Kuppuswamy scale,[10] doing exercise, the smoking status, and the body mass index (BMI), were collected through personal interviews with the patients. Section 2: Details of medical conditions, including systolic and diastolic blood pressure, renal biomarkers, like eGFR and UACR, and other biochemical values, like glycated hemoglobin (HbA1c), total cholesterol, triglycerides, and hs-CRP, were retrieved from the patients' medical records. Section 3: The clinical examination was carried out by a trained examiner to assess periodontal parameters, including the oral hygiene status assessed with the Simplified Oral Hygiene Index (OHI-S),[11] PPD, the position of the gingival margin in terms of relative clinical attachment loss (CAL), and bleeding on probing (BoP) measured at 6 different sites (mesiobuccal, distobuccal, buccal, palatal/lingual, mesiolingual, and distolingual) for each tooth using a pressure-sensitive probe (Bluedent India, Chennai, India) with a standardized pressure of 20 g. The periodontal inflamed surface area (PISA), which reflects the amount of inflamed periodontal tissue in square millimeters, was calculated for each patient by entering the PPD, CAL and BoP measurements into a spreadsheet accessed via www.parsprototo.info.[4]

High-sensitivity assay techniques, such as immunonephelometry, immunoturbidimetry, high-sensitivity enzyme-linked immunosorbent assay (ELISA), and resonant acoustic profiling (RAP), can detect CRP with a sensitivity range of 0.01–10 mg/L. In the present study, immunoturbidimetry was used, in which the immune complexes formed in the solution scatter light in proportion to their size, shape and concentration. Turbidimeters measure the reduction of incidence light due to reflection, absorption or scatter. What is measured in this procedure is the rate of decrease in the intensity (increase in the absorbance) of the light transmitted through the particles suspended in the solution, which is due to the complexes being formed during the immunological reaction between CRP in the patient's serum and the rabbit anti-CRP antibodies coating the latex particles. For this method, the AU400/400e/480, AU600/640/640e/680 and AU2700/5400 analyzers (Beckman Coulter, Brea, USA) were used with reagents, including glycine buffer (100 mmol/L) and latex particles coated with rabbit anti-CRP antibodies (<0.5%).

All data related to medical conditions and periodontal parameters was assessed at 3 different time points, i.e., at baseline (before any interventions), at 3 months and at 6 months.

Statistical analysis

After data collection, the data was encoded and entered into Microsoft® Excel® 2019 (Microsoft Corporation, Redmond, USA). The data was checked for normality with the Shapiro–Wilk test. As the data deviated significantly from a normal distribution ($p < 0.05$), non-parametric tests were applied for the analysis. Demographic variables were reported as mean (M) and standard deviation (SD) or as number (n) and percentage (%), and compared using the χ^2 test. Student's t test was used to compare the mean values for quantitative data. The data for clinical variables

was presented as *M* and *SD* or as median (*Me*) and interquartile range (*IQR*). The values for different time points were compared within both groups using Friedman's test. In case of significant results of Friedman's test, Scheffe's test was used for post hoc pairwise comparisons. The Mann–Whitney *U* test was applied for comparisons between the 2 groups. For all tests, the level of statistical significance was set at $p < 0.05$. The statistical analysis was performed using the IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA).

Results

Out of the total 100 patients selected for this study, 80 completed 6 months of follow-up. During the 6-month follow-up period, 14 patients developed ESRD and required preparation for dialysis (the creation of an arteriovenous (AV) fistula), 4 died during the course of treatment, and 2 did not attend follow-up. Therefore, 47 patients from group 1 and 33 patients from group 2 completed the full study (Fig. 1, Table 1).

Demographic characteristics for groups 1 and 2 are shown in Table 1. The mean patient age was 50.62 ±10.35 years and 50.45 ±10.87 years in group 1 and group 2, respectively. There were no statistically significant differences in demographic variables between the groups ($p > 0.05$).

There were highly significant differences within groups 1 and 2 when the median values of periodontal parameters, systemic inflammatory marker hs-CRP and renal biomarkers were compared across the study time points ($p < 0.001$). The intragroup comparisons with regard to different time points also exhibited significant differences in the post hoc test ($p < 0.05$) (Table 2).

Table 1. Demographic characteristics and some clinical data of the study participants

Variables			Group 1 ($n = 47$)	Group 2 ($n = 33$)	*p*-value
Age[a] [years]			50.62 ±10.35	50.45 ±10.87	0.950
Gender[b]	male		35 (43.8)	24 (30.0)	1.000
	female		12 (15.0)	9 (11.3)	
SES[c]	upper lower		4 (5.0)	1 (1.3)	0.070
	lower middle		11 (13.8)	8 (10.0)	
	upper middle		25 (31.3)	24 (30.0)	
	upper		7 (8.80)	0 (0.0)	
Doing exercise[b]	yes		8 (10.0)	12 (15.0)	0.080
	no		39 (48.8)	21 (26.3)	
Smoking[c]	never		23 (28.8)	19 (23.8)	0.750
	former		19 (23.8)	11 (13.8)	
	current		5 (6.3)	3 (3.8)	
Comorbidity[b]	diabetes	yes	21 (26.3)	11 (13.8)	0.430
		no	26 (32.5)	22 (27.5)	
	CVS disorder	yes	11 (13.8)	9 (11.3)	0.890
		no	36 (45.0)	24 (30.0)	
	CNS disorder	yes	2 (2.5)	0 (0.0)	0.640
		no	45 (56.3)	33 (41.3)	
Blood pressure[a] [mm Hg]	systolic		140.64 ±15.89	142.42 ±22.36	0.680
	diastolic		86.21 ±9.61	86.97 ±10.75	0.690
PISA[d] [mm^2] (at baseline)			1,772.70 ±580.06	1,785.98 ±611.90	0.900
BMI[a] [kg/m^2]			23.97 ±3.88	23.23 ±3.83	0.410
eGFR[d] [mL/min/1.73 m^2] (at baseline)			17.74 ±7.80	19.30 ±9.56	0.580

Data presented as mean ± standard deviation (*M* ±*SD*) or as number (percentage) (*n* (%)). SES – socio-economic status assessed by the Kuppuswamy scale[10]; CVS – cardiovascular system; CNS – central nervous system; PISA – periodontal inflamed surface area; BMI – body mass index; eGFR – estimated glomerular filtration rate; [a] *t* test; [b] χ^2 test with a continuity correction; [c] χ^2 test; [d] Mann–Whitney *U* test.

Table 2. Comparison of clinical variables within the groups across the study time points

Group	Variables	Time point	min–max	*M* ±*SD*	*Me* (*IQR*)	*p*-value†
Group 1	hs-CRP [mg/L]	at baseline	0.34–119.38	6.59 ±17.25[b,c]	3.42 (2.99)	<0.001**
		at 3 months	0.42–29.30	3.90 ±4.80[a,c]	2.80 (2.09)	
		at 6 months	0.56–15.49	3.03 ±3.20[a,b]	2.10 (1.43)	
	eGFR [mL/min/1.73 m^2]	at baseline	7.00–35.00	17.74 ±7.80[b,c]	16.00 (12.00)	<0.001**
		at 3 months	8.00–39.00	20.59 ±8.21[a,c]	20.00 (12.00)	
		at 6 months	8.00–57.00	25.04 ±11.53[a,b]	23.00 (18.00)	
	UACR [mg/gCr]	at baseline	10.00–500.00	184.68 ±209.99[b,c]	150.00 (490.00)	<0.001**
		at 3 months	10.00–500.00	169.57 ±131.71[a]	75.00 (140.00)	
		at 6 months	10.00–500.00	138.40 ±143.69[a]	75.00 (125.00)	
	PISA [mm^2]	at baseline	1,031.12–3,506.00	1,772.70 ±580.06[b,c]	1,679.54 (617.30)	<0.001**
		at 3 months	132.45–690.12	279.82 ±119.05[a,c]	245.33 (156.19)	
		at 6 months	100.21–598.65	249.46 ±92.72[a,b]	116.43 (90.27)	
Group 2	hs-CRP [mg/L]	at baseline	0.57–16.59	4.80 ±4.22[b,c]	3.58 (2.38)	<0.001**
		at 3 months	0.46–20.90	5.88 ± 5.12[a,c]	3.98 (4.19)	
		at 6 months	0.44–32.23	6.79 ±6.49[a,b]	4.32 (6.43)	
	eGFR [mL/min/1.73 m^2]	at baseline	6.00–42.00	19.30 ±9.56[b,c]	18.00 (8.50)	<0.001**
		at 3 months	6.00–53.00	17.81 ±11.10[a,c]	14.00 (10.00)	
		at 6 months	4.00–45.00	16.06 ±9.39[a,b]	15.00 (9.50)	
	UACR [mg/gCr]	at baseline	10.00–500.00	191.52 ±204.86[b,c]	75.00 (475.00)	<0.001**
		at 3 months	10.00–500.00	205.00 ±195.49[a]	150.00 (425.00)	
		at 6 months	10.00–500.00	218.94 ±202.29[a]	150.00 (425.00)	
	PISA [mm^2]	at baseline	1,062.85–3,564.15	1,785.98 ±611.90[b,c]	1,587.84 (615.08)	<0.001**
		at 3 months	1,150.21–3,523.10	1,819.25 ±599.46[a,c]	1,647.89 (662.13)	
		at 6 months	1,190.22–3,594.25	1,854.32 ±593.27[a,b]	1,696.33 (658.95)	

min – minimum; max – maximum; *M* – mean; *SD* – standard deviation; *Me* – median; *IQR* – interquartile range; hs-CRP – high-sensitivity C-reactive protein; UACR – urine albumin-to-creatinine ratio; [a] at baseline; [b] at 3 months; [c] at 6 months; † Friedman's test; ** highly significant ($p < 0.001$). The 95% Monte Carlo confidence interval (*CI*) for group 1 was 0.00–0.06 and for group 2 it was 0.00–0.09. Post hoc Scheffe's test applied for pairwise comparisons showed significant differences at $p < 0.05$.

Table 3 and Fig. 2–5 show the intergroup comparisons of the median values of periodontal parameters, hs-CRP and renal biomarkers between the 2 groups at different time points. No statistically significant differences were observed when periodontal parameters, hs-CRP and renal biomarkers were compared between the 2 groups at baseline ($p > 0.05$). However, the above-mentioned variables were statistically significantly different in the 2 groups at 3-month and 6-month follow-up ($p < 0.05$).

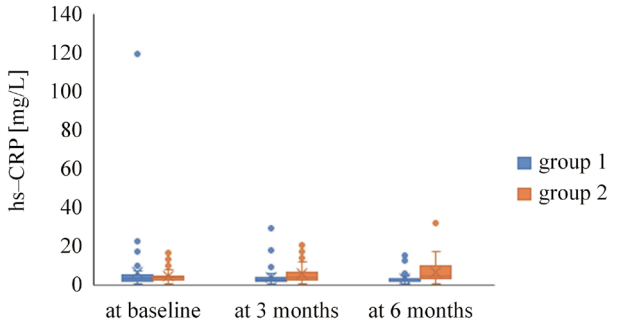

Fig. 2. Comparison of high-sensitivity C-reactive protein (hs-CRP) between the groups according to the time points

Data presented as *M* ±*SD*.

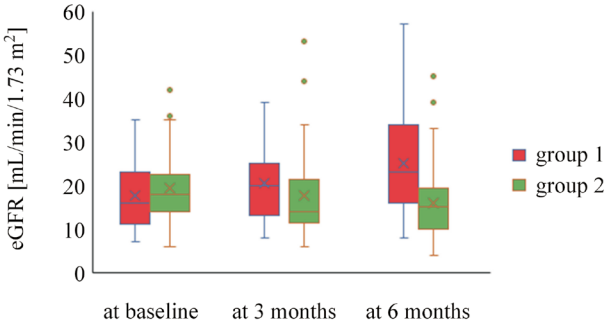

Fig. 3. Comparison of the estimated glomerular filtration rate (eGFR) between the groups according to the time points

Data presented as *M* ±*SD*.

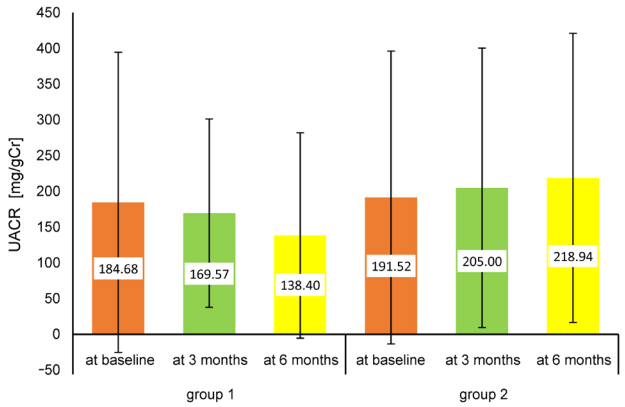

Fig. 4. Comparison of the urine albumin-to-creatinine ratio (UACR) between the groups according to the time points

Data presented as *M* ±*SD*.

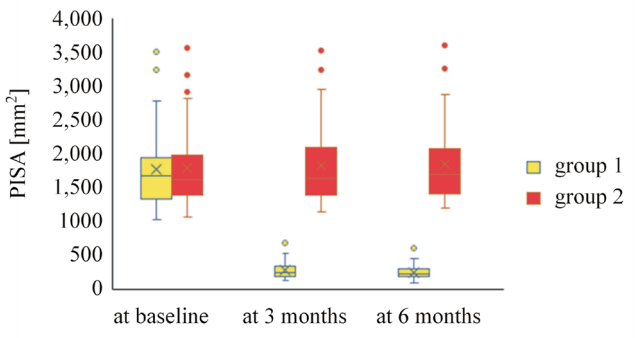

Fig. 5. Comparison of the periodontal inflamed surface area (PISA) between the groups according to the time points

Data presented as *M* ±*SD*.

Table 3. Comparison of clinical variables between the groups across the study time points

Variables	Time point	Group	*M* ±*SD*	95% *CI*	*p*-value†
hs-CRP [mg/L]	at baseline	group 1	6.59 ±17.25	0.74–0.91	0.880
		group 2	4.80 ±4.22		
	at 3 months	group 1	3.90 ±4.80	0.00–0.01	0.010*
		group 2	5.88 ±5.12		
	at 6 months	group 1	3.03 ±3.20	0.00–0.04	<0.001**
		group 2	6.79 ±6.49		
eGFR [mL/min/1.73 m²]	at baseline	group 1	17.74 ±7.80	0.34–0.59	0.580
		group 2	19.30 ±9.56		
	at 3 months	group 1	20.59 ±8.21	0.00–0.06	0.030*
		group 2	17.81 ±11.10		
	at 6 months	group 1	25.04 ±11.53	0.00–0.04	<0.001**
		group 2	16.06 ±9.39		
UACR [mg/gCr]	at baseline	group 1	184.68 ±209.99	0.73–0.89	0.810
		group 2	191.52 ±204.86		
	at 3 months	group 1	169.57 ±131.71	0.01–0.12	0.030*
		group 2	205.00 ±195.49		
	at 6 months	group 1	138.40 ±143.69	0.00–0.04	<0.001**
		group 2	218.94 ±202.29		
PISA [mm²]	at baseline	group 1	1,772.70 ±580.06	0.85–0.97	0.900
		group 2	1,785.98 ±611.90		
	at 3 months	group 1	279.82 ±119.05	0.00–0.04	<0.001**
		group 2	1,819.25 ±599.46		
	at 6 months	group 1	249.46 ±92.72	0.00–0.04	<0.001**
		group 2	1,854.32 ±593.27		

† Mann–Whitney *U* test; * statistically significant ($p < 0.05$); ** highly significant ($p < 0.001$).

Discussion

A recent systematic review on the association between CP and CKD, and the effects of periodontal treatment concluded that there was an increased incidence of CP in CKD patients, with odds ratios (*OR*s) ranging from 1.49 to 2.39.[12] After multivariable adjustment, it was also found that CP was associated with CKD and that it was a modifiable

risk factor for CKD. The authors thus emphasized the need for carefully designed interventional studies to determine whether periodontal treatment can reduce the incidence and/or severity of CKD.[12]

The present study was conducted to evaluate the effect of periodontal treatment on the systemic inflammatory response. The results showed a causal association between the treatment of CP and high serum levels of hs-CRP as well as high values of eGFR and UACR in CKD patients. However, there were many confounding factors, like hypertension, diabetes, smoking, and SES, leading to an increase in the systemic inflammatory burden.

In the present study, hs-CRP was used to assess the local inflammatory processes leading to the systemic inflammatory burden. It has been documented that CP induces an acute-phase inflammatory response that can be measured based on the serum level of hs-CRP, which is regarded as a key biomarker of systemic inflammation.[13] High-sensitivity CRP is mainly synthesized by hepatocytes in the liver in response to inflammation and tissue damage, but can also be produced locally by arterial tissue, and is regulated by cytokines, like IL-6, IL-1β and tumor necrosis factor alpha (TNF-α).[14]

For hs-CRP, 1–3 mg/L is considered the critical range pertaining to risk prediction. Elevated levels of hs-CRP (>2.1 mg/L) are associated with a higher incidence of acute thrombotic events, including stroke and myocardial infarction.[15]

According to a systematic review,[16] there is convincing evidence that CRP is chronically elevated in CP patients as compared to healthy controls and that it is affected by the severity of CP, with benefits of periodontal treatment for such patients as compared to untreated ones.[17]

In the present study, the serum levels of hs-CRP in group 1 decreased after NSPT from 6.59 ±17.25 mg/L (at baseline) to 3.90 ±4.80 mg/L (at 3 months), and then to 3.03 ±3.20 mg/L (at 6 months). By contrast, in group 2, the serum levels of hs-CRP increased from 4.80 ±4.22 mg/L (at baseline) to 5.88 ±5.12 mg/L (at 3 months), and then to 6.79 ±6.49 mg/L (at 6 months). D'Aiuto et al. reported that the serum levels of CRP were reduced in 65 patients with severe CP, otherwise systemically healthy, who were subjected to intensive NSPT for 2 months.[18] Vilela et al. reported a reduction in the levels of prohepcidin, IL-6 and hs-CRP in both CKD and control patients following periodontal treatment.[19] Their findings suggest that CP induces the systemic inflammatory response and is more severe in CKD patients; however, successful periodontal treatment reduces the inflammatory burden in such patients, and thus may be a beneficial intervention in the therapy of CKD.[19] In a study by Guo and Lin, 53 patients were divided into 3 groups: group A received treatment for both CKD and CP; group B received treatment for CKD only; and group C received treatment for CP only.[20] The authors concluded that in patients with CKD and CP, NSPT decreased both the clinical parameters of CP and the serum markers of systemic inflammation. The levels of hs-CRP were reduced in all groups, especially in group A, with a decrease from 4.71 ±1.55 mg/L pre-operatively to 3.70 ±1.28 mg/L post-operatively.[20] Elevated levels of CRP are also related to several other CKD risk factors, such as SES, old age, prolonged diabetes, CVD, and albuminuria; however, periodontitis is an independent predictor of increased hs-CRP in CKD patients.[21] Considering the presence of similar confounding factors in both groups in the present study, a decrease in hs-CRP in group 1 can be attributed to NSPT only, wherein patient motivation and counseling by the periodontist during treatment led to psychological benefits, an increased patient understanding of the need to maintain good oral hygiene, and increased treatment compliance with regard to both CKD and CP.

In this study, eGFR and UACR were used as renal biomarkers to evaluate the progression or regression of CKD. There are many methods to diagnose and stage CKD. The estimated GFR is used for diagnosis, staging and prognosis. It also plays an important role in drug dosing and risk stratification for clinical procedures and future outcomes. The estimated GFR is usually calculated based on the plasma creatinine values, using the Modification of Diet in Renal Disease (MDRD) Study equation or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[22] In the present study, the MDRD formula was used to calculate eGFR. This formula has been extensively validated in multiple samples with and without CKD. Many of the studies have shown a good performance of the method in CKD patients, including those with diabetes or kidney transplants, and the elderly.[23] Albuminuria is the most frequently used marker of kidney damage in clinical practice and research. Over 50% of CKD cases may be missed if UACR is ignored. The estimation of UACR has advantages over other tests in terms of sensitivity and the level quantification. Moreover, the assessment of UACR in CKD screening appears to enable a more accurate prediction of poor quality of life.[24]

The results of the present study are similar to those obtained by Almeida et al.[25] The authors reported significant improvement after an intervention in the median (25%, 75% percentiles) eGFR values from 34.6 (27.0, 44.7) mL/min/1.73 m^2 at baseline to 37.6 (29.7, 57.0) mL/min/1.73 m^2 on day 90 and 37.6 (28.6, 56.0) mL/min/1.73 m^2 on day 180.[25]

Carillo Artese et al. evaluated the effect of NSPT with no antibiotics on the levels of creatinine and eGFR at baseline and 3 months after treatment in 21 pre-dialysis CKD patients and 19 individuals without CKD.[26] Both groups showed significant and similar post-treatment improvement in all parameters examined.[26] Periodontal treatment may thus play an important role in reducing inflammatory mediators. Several studies have indicated that the kidneys are important for the clearance of cytokines.[27] High levels of inflammatory markers may have detrimental effects on the renal system, because an increased nephron filtration

of plasma proteins results in further release of cytokines in the renal interstitium, with subsequent fibroblast proliferation, fibrogenesis, and ultimately renal scarring, contributing to progressive deterioration in function. Hence, periodontal treatment could have positive effects on both systemic inflammatory markers and renal endothelial function via more effective filtration.[28]

The present study used PISA as the main periodontal parameter. Periodontal inflammation, which occurs locally, but can disseminate systemically, is the biological basis for the plausibility of any potential association between CP and other diseases. In this sense, any classification of CP as a potential risk factor for any systemic condition should quantify the inflammatory burden posed by periodontitis.[29] For this reason, the PISA classification, which was developed in 2008, is based not only on linear measurements, such as CAL and gingival recession (GR), but also on BoP. Thus, PISA reflects the surface area of bleeding pocket epithelium in square millimeters.[4] Notably, PISA is considered by medical professionals other than dentists as a useful index for understanding the degree of inflammation induced by PD. As stated by Leira et al., a PISA value ≥130.33 mm^2 indicates CP while values ranging from 934.71 mm^2 to 3,274.96 mm^2 indicate severe periodontitis cases according to the Centers for Disease Control and Prevention and American Academy of Periodontology (CDC-AAP) classification.[29] A study evaluating the dose–response relationship between PISA and the level of HbA1c revealed that PISA indeed appeared to be a valuable tool for assessing the relationships between the amount of inflamed periodontal tissue and well-defined disease activity parameters, such as HbA1c.[30] To date, one Japanese study has used this index in patients with CKD and CP.[31] The study investigated the effect of PD on kidney function in community-dwelling elderly patients. Participants were classified into quartile groups according to their PISA score, and then divided into 2 groups: the highest quartile vs. the other 3 groups combined. The results showed that patients in the highest PISA quartile had a 2.6-fold greater risk of a decreased kidney function after 2 years of follow-up (*OR*: 2.58; 95% confidence interval (*CI*): 1.34–4.98).[31]

The results of the present study differ from those of the Kidney and Periodontal Disease (KAPD) study.[32] In the KAPD study, there was no change in the magnitude of the biomarkers measured in response to intensive periodontal treatment. There are multifactorial potential reasons for this difference, including the presence of more severe PD in the immediate treatment group, the lack of a true control group, and the fact that the rescue group in the KAPD study received oral hygiene instructions, cleaning above the gum line, and had hopeless teeth extracted, all of which could have reduced the burden of PD, thus reducing the ability to achieve a clear separation in terms of periodontal status between groups and to detect differences in biomarkers.[32]

Persistent low-grade inflammation has been recognized as an essential component of CKD, and so CP may contribute to this inflammation. The pathogenesis of CP involves polymicrobial synergy and dysbiosis (the PSD model), leading to a greater relative abundance of individual components of the bacterial community as compared to their abundance in health, with alterations in the host–microbe crosstalk sufficient to mediate destructive inflammation and bone loss.[33] Among all periodontal pathogens, the role of *Porphyromonas gingivalis* (*P. gingivalis*) is the most powerful in this respect. Hence, in PD, a larger area of the inflamed periodontal surface may allow this keystone periodontal pathogen and its byproducts, like gingipains and fimbriae, to enter circulation through an ulcerated periodontal pocket, inducing bacteremia.[34] In turn, this appears to activate cells, series of receptors and signaling pathways, e.g., dendritic cells, which are the critical cells of the immune response and the 'presenters' of antigens that can also serve as the 'transporters' of bacteria and their virulence factors.[34] Moreover, gingipains from *P. gingivalis* have been shown to activate protease-activated receptors (PARs) and toll-like receptors (TLRs), leading to an increased inflammatory response with the release of chemokines[35] and pro-inflammatory cytokines, like IL-6, prostaglandin E2 (PGE2) and thromboxane B2 (TXB2), in the kidneys, generating an inflammatory cascade that can lead to kidney dysfunction. These factors may accelerate angiogenesis, thrombus formation and platelet aggregation via several mechanisms causing vasoconstriction and a chronic reduction in renal blood flow. The atherogenesis of the renal artery and arterioles can also cause ischemia, glomerular sclerosis and severe renal insufficiency.[36]

Alternatively, the effect of periodontitis on renal tissue can be explained by the concept of oral microbial pathogens affecting distant organs directly through bloodstream dissemination, a 'mobile oral microbiome', involving the direct migration of resident oral microbial species and their colonization in distant organs.[37] In some case studies,[38] these oral pathogens have been directly indicated as a cause of bacterial endocarditis, acute post-streptococcal glomerulonephritis and infective endocarditis-associated glomerulonephritis. The *P. gingivalis* fimbriae bind to cluster of differentiation 14 (CD14) and activate TLR2-phosphoinositide 3-kinase (PI3K) signaling. The activated TLR2 pro-adhesive signaling pathway enhances leukocyte–endothelial cell interactions, transendothelial migration and the interaction of its cell surface fimbriae with complement receptor 3 (CR3). The activated CR3 then downregulates IL-12p70, a key cytokine involved in macrophage phagocytosis. This CR3-associated reduction in IL-12p70 impairs the clearance of *P. gingivalis* and promotes its survival in renal tissue.[39]

At the molecular level, hsa-miR-146a, hsa-miR-146b, hsa-miR-155, and hsa-miR200 could be considered as potential biomarkers of the progression of PD.[40] To date, only an animal study with transcription factor

signal transducer and activator of transcription (STAT) has been carried out considering both PD and CKD.[41] The study demonstrated that STAT1 expression was augmented by periodontitis, and resulted in the upregulation of inflammatory and fibrosis genes, which aggravated hypertensive renal injuries in the mouse model, thus suggesting a new target for periodontal treatment in CKD patients.[41]

Notably, 30–50% of both ESRD patients on RRT and non-dialyzed CKD patients show evidence of an active systemic inflammatory response in the form of elevated serum levels of CRP and other pro-inflammatory cytokines, like IL-1 and IL-6.[42] Therefore, there are 2 potential mechanisms through which the immune system and the inflammatory process may affect distant organs, including the kidneys. However, the mechanisms through which CP influences CKD are not well understood. This process is not likely to involve a single causal mechanism, but rather a combination of mechanisms.

The present study is a novel effort to evaluate the effect of NSPT on the PISA scores in patients with CKD and CP. An increase in the PISA scores, as observed in group 2 in our study, can be explained by the high prevalence and severity of periodontitis in CKD patients. An in-depth review by Akar et al. explored the etiological factors and systemic consequences of poor oral health in CKD patients, and observed that oral hygiene was generally poor in such patients, which might cause inflammation, infection and atherosclerosis, and could worsen their condition.[43] Hence, maintaining good oral health is essential for such patients, and should be monitored both by nephrologists and periodontists.

Apart from NSPT, periodontal therapy can include laser therapy (LT) and photodynamic therapy (PDT) as methods of antimicrobial treatment to kill periodontal bacteria with few adverse effects. The abovementioned alternatives can be used as independent treatment or as an adjunct to SRP. A randomized clinical trial evaluating all 3 treatment modalities reported changes in the expression of 13 different cytokines, i.a., IL-1 and IL-6, and 9 acute-phase proteins, i.a., CRP, in the gingival crevicular fluid (GCF) of CP patients.[44]

The present study is the pilot study of an ongoing longitudinal prospective trial, wherein the correlation between CKD and the treatment of CP will be evaluated through the multivariate logistic regression analysis. Some limitations of the present study are as follows: the patient population was taken from a single center; some patients dropped out; and the frequency of use of antihypertensive drugs and their effects on renal biomarkers were not examined. These limitations should be addressed in future studies.

Conclusions

Chronic periodontitis is a non-traditional risk factor for CKD that can be treated in a dental office by means of NSPT and proper maintenance visits, which are expected to reduce the inflammatory burden, and thus limit the effect of contributing factors on further progression of CKD. Therefore, methods of early CP detection, risk factors for kidney disease and specifically dental interventions warrant attention from global public health organizations. In the future, larger studies using different biomarkers and long-term follow-up are needed to evaluate the contribution of periodontal treatment in reducing the risk of CKD or its progression.

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Influence of orthodontic brackets and permanent retainers on the diagnostic image quality of MRI scans: A preliminary study

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Abstract

Background. Orthodontic treatment with fixed mechanotherapy using appliances and permanent retainers bonded after treatment is a routine procedure performed in clinical dentistry. Patients with braces or retainers sometimes need to undergo magnetic resonance imaging (MRI) for various reasons. Radiologists do not know the exact impact of the materials used in orthodontics on the diagnostic image quality of MRI scans.

Objectives. The aim of the study was to evaluate the influence of different types of orthodontic brackets and permanent retainers on the diagnostic image quality of MRI scans.

Material and methods. Twenty patients with bonded brackets (stainless steel buccal/labial, stainless steel lingual, ceramic self-ligating with metal slots, ceramic, and polycarbonate) and 18 patients with bonded fixed retainers (titanium, fiber-reinforced composite, multi-stranded stainless steel, and different combinations of permanent retainers) participated in the study. The same adhesive was used for bonding. Cranial MRI scans of 6 regions were acquired for each subject, using a 1.5T MAGNETOM machine. Six radiologists evaluated the images and provided scores based on the modified receiver operating characteristic (ROC) analysis of distortion. The paired Wilcoxon signed-rank test was used to assess differences between the materials and the anatomic sites with regard to the distortion rating scale. Cohen's kappa coefficient (κ) was applied to establish the interrater reliability.

Results. A statistically significant difference was found between stainless steel brackets (both buccal/labial and lingual) and all other experimental materials in terms of mean distortion scores ($p = 0.020$ or $p = 0.024$). The interrater reliability proved to be high.

Conclusions. Stainless steel buccal/labial and lingual brackets caused maximum distortion of the images, which became non-diagnostic; hence, such brackets should be removed before MRI. Ceramic and polycarbonate brackets as well as fiber-reinforced composite retainers did not distort the images; thus, they need not be removed. Ceramic self-ligating brackets with metal slots, titanium retainers, multi-stranded stainless steel retainers, and combinations of fixed retainers caused minimal distortion; however, the images were still diagnostic. Hence, patients using these materials may not need to have them removed before MRI.

Keywords: magnetic resonance imaging, orthodontic brackets, bonded retainers, MRI distortion

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Introduction

Magnetic resonance imaging (MRI) is one of the most powerful diagnostic tools in radiology. It is characterized by high sensitivity and specificity. Besides, it is a non-invasive procedure. The system does not use ionizing radiation, and for the majority of people there is little risk associated with the application of the magnetic field. This radiological modality is indispensable when investigating soft tissue tumors, including those of the head and neck,[1] temporomandibular joint (TMJ) pathology,[2] cardiovascular pathology,[3] seizures,[4] and cerebral palsy.[5] The number of MRI scans performed per year is steadily increasing.

Fixed orthodontic treatment is a standard and increasingly prescribed practice for both children and adults. It involves bonding orthodontic brackets in the patient's mouth for the duration of treatment, which generally lasts 24–30 months.[6] Orthodontic treatment is routinely followed by long-term retention to maintain the treatment result with the use of a metal or non-metal wire that is fixed to the lingual surface of the lower anterior teeth and remains in situ for several years.[7]

Patients with fixed multibracket orthodontic appliances sometimes require the MRI examination of the head and neck region for various reasons. These may include diagnosing epilepsy, recurrent headaches, trauma, and the pathology of the salivary glands, tongue and TMJ. While it is known that fixed multibracket orthodontic appliances cause MRI artifacts, the extent and severity of image loss is not clearly described in the literature. The heating of the appliance which occurs during MRI, although negligible, is the reason why it may be necessary to remove the orthodontic appliances or archwires to prevent artifacts.[8,9] This increases the financial and biological burdens, since the removal and later reinstallation of fixed orthodontic appliances are costly, labor-intensive[10] and time-consuming, and require the remodeling of the bone once again. However, the removal of archwires is easy and less time-consuming.[11]

A few studies have reported that the artifacts caused by stainless steel brackets and wires do result in some distortion of the MR images of the brain; however, these images remained diagnostic.[12,13] On the other hand, other studies have reported that orthodontic appliances can render TMJ and brain MR images non-diagnostic.[14] There is a lack of consensus regarding the best practice protocols to employ for patients who need MRI during the period of orthodontic treatment or retention with fixed appliances. There are, however, guidelines from the Polish Orthodontic Society, the Polish Medical Radiological Society and the Polish Dental Association.[15] Most of the studies regarding stainless steel brackets were performed on skulls and in volunteers for whom MRI was used without bonding the brackets on the teeth, but instead incorporating them in vacuum-formed sheets, which are then worn by volunteers. Moreover, the effects of some recently introduced brackets, such as ceramic self-ligating brackets with metal slots, ceramic brackets, polycarbonate brackets, and different types of lingual retainers on the diagnostic image quality of MRI scans have not been researched. Although there are guidelines[15] and literature regarding MRI artifacts that are due to brackets and some wires, this may be the first comprehensive in vivo study to evaluate the diagnostic image quality of MRI scans in patients with different types of orthodontic brackets and fixed retainers. Therefore, this study aimed to evaluate the influence of different types of orthodontic brackets and permanent retainers bonded in orthodontic patients on the diagnostic image quality of MRI scans.

Material and methods

Ethical statement

This research was conducted after obtaining approval from the institutional ethics committee at Kamineni Institute of Dental Sciences, Sreepuram, Narketpally, India (KIDS/IEC/2015/22). The study was carried out following the standards set out in the Declaration of Helsinki for experiments involving humans. Informed consent was obtained from all subjects, who voluntarily participated in the study. They were healthy patients in the age range of 18–25 years.

Patient selection

Thirty-eight subjects who met the following inclusion criteria were recruited for the study: no metal medical devices, like aneurysmal clips and pacemakers; no metal dental fillings, metal-containing crowns or dental implants; no pregnancy; and no requirement for sedation during MRI. All 38 subjects who volunteered to participate in the study were about to undergo fixed orthodontic treatment or about to start the retention phase. The purpose of the study, including its possible risks, was explained to the participants.

The types of brackets/retainers and the corresponding numbers of patients who underwent MRI were as follows:

- stainless steel buccal/labial brackets for all teeth erupted, from anterior ones to second premolars in both the maxillary and mandibular arch, without molar bands and wires (Unitek™ Gemini; 3M Unitek, Monrovia, USA): 4 patients;
- stainless steel lingual brackets for all teeth erupted, from anterior ones to second premolars in both the maxillary and mandibular arch, without molar bands and wires (Invisible 7G; Classic Orthodontics, Stafford, USA): 4 patients;
- ceramic self-ligating brackets with metal slots made of stainless steel for all teeth erupted, from anterior ones to second premolars in both the maxillary and mandibular arch, without molar bands and wires (Unitek Clarity SL; 3M Unitek): 4 patients;
- ceramic brackets for all teeth erupted, from anterior ones to second premolars in both the maxillary and mandibular arch, without molar bands and wires (Inspire ICE; Ormco, Brea, USA): 4 patients;

– polycarbonate brackets for all teeth erupted, from anterior ones to second premolars in both the maxillary and mandibular arch, without molar bands and wires (Ora-plus; JJ Orthodontics, Munipara, India): 4 patients;
– titanium retainers bonded from right canine to left canine in the mandibular arch only (Ortho-Direct, West Columbia, USA): 4 patients;
– fiber-reinforced composite retainers bonded from right canine to left canine in the mandibular arch only (everStick® ORTHO; GC America, Alsip, USA): 4 patients;
– multi-stranded stainless steel retainers in the maxillary and mandibular arches: 4 patients;
– combinations of these fixed retainers bonded from right canine to left canine in the maxillary and mandibular arches, including:
  • a stainless steel retainer in the maxillary arch and a titanium retainer in the mandibular arch: 3 patients,
  • a stainless steel retainer in the maxillary arch and a fiber-reinforced composite retainer in the mandibular arch: 3 patients.

Thus, a total of 38 subjects (20 with different brackets and 18 with different retainers) participated in the study.

MRI protocol

A 1.5 Tesla Siemens MAGNETOM MRI machine (Siemens, Erlangen, Germany) with a 12-channel head coil was used.

Six regions of interest (ROI) were analyzed: TMJ; the tongue; the maxilla; the mandible; the maxillary sinus; and the posterior cerebral fossa.

For the posterior cerebral fossa, the MRI brain protocol was used; it is shown in Table 1.

Diffusion-weighted (DW) images were acquired with a single-shot spin-echo echo planar imaging (SE-EPI) sequence (repetition time (TR)/echo time (TE): 5,000 ms/100 ms) using 3 values of 0 s/mm^2, 500 s/mm^2 and 1,000 s/mm^2, with a slice thickness of 5 mm, an inter-slice gap of 1.5 mm, a field of view (FoV) of 240 mm × 240 mm, and a matrix of 128 × 128. Apparent diffusion coefficient (ADC) maps were generated from the DW image datasets. For susceptibility-weighted imaging (SWI), TR/TE was 6,400 ms/30 ms, with a slice thickness of 5 mm, an inter-slice gap of 1.5 mm, an FoV of 240 mm × 240 mm, and a matrix of 256 × 256.

For TMJ, sagittal, axial and coronal proton density (PD)-weighted and turbo spin-echo (TSE) transverse relaxation time (T2)-weighted sequences were employed with TR 5,653 ms, TE 13 ms and 102 ms, a slice thickness of 2 mm, an inter-slice gap of 0.2 mm, an FoV of 160 mm × 160 mm, and a matrix of 256 × 256. For the remaining regions, the MRI neck protocol was used. Axial and coronal TSE T2-weighted images were acquired with TR 5,600 ms, TE 114/7 ms, a slice thickness of 5 mm, an inter-slice gap of 0.5 mm, an FoV of 240 mm × 240 mm, and a matrix of 320 × 320.

No paramagnetic agent was used, as there was no indication for the use of a paramagnetic agent.

Magnetic resonance imaging scans were taken for all 38 subjects to study diagnostic image quality. Since the orthodontic appliances were securely bonded with orthodontic composite, there were no special precautions taken regarding the possible dislodgement and heating of the appliances, as previous studies had found changes in the temperature of the appliances to be clinically insignificant (within 1°C).[16] The MRI scans for all types of bracket and retainer materials are shown in Fig. 1–10. Non-diagnostic or unclear ROI are marked with arrows. None of the patients reported any discomfort or pain during MRI examination. The MRI scans were analyzed by a panel of 6 qualified and licensed radiologists, who assessed their diagnostic quality. The scans were compared with controls that included images from the archives of the Department of Radio-Diagnosis of Kamineni Institute of Medical Sciences. The radiologists ranked the images according to the distortion observed in the abovementioned ROI, using the modified receiver operating characteristic (ROC) analysis of distortion scoring system (Table 2), as described by Elison et al.[17] In this method of distortion classification, a score of 3 represents the cut-off point for clinical usability. Images with a score of 3 have moderate distortion or artifacts, but they can still be used for diagnosis.

Table 2. Modified receiver operating characteristic (ROC) analysis of distortion scoring system

Score	Image appearance	Inference
1	no distortion or artifacts	diagnostic
2	minimal distortion or artifacts	diagnostic
3	moderate distortion or artifacts	moderately diagnostic
4	severe distortion	non-diagnostic

Table 1. Magnetic resonance imaging (MRI) protocol for the posterior cerebral fossa

Sequence	TR/TE/TI [ms]	Slice thickness [mm]	Inter-slice gap [mm]	FoV [mm^2]	Matrix
Axial TSE T1-weighted	5,000/14	5	1.5	240 × 240	256 × 256
Axial TSE T2-weighted	4,000/102	5	1.5	240 × 240	256 × 256
Axial FLAIR	9,000/84/2,500	5	1.5	240 × 240	256 × 256

TR – repetition time; TE – echo time; TI – inversion time (for FLAIR); FoV – field of view; TSE – turbo spin-echo; T1 – longitudinal relaxation time; T2 – transverse relaxation time; FLAIR – fluid-attenuated inversion recovery.

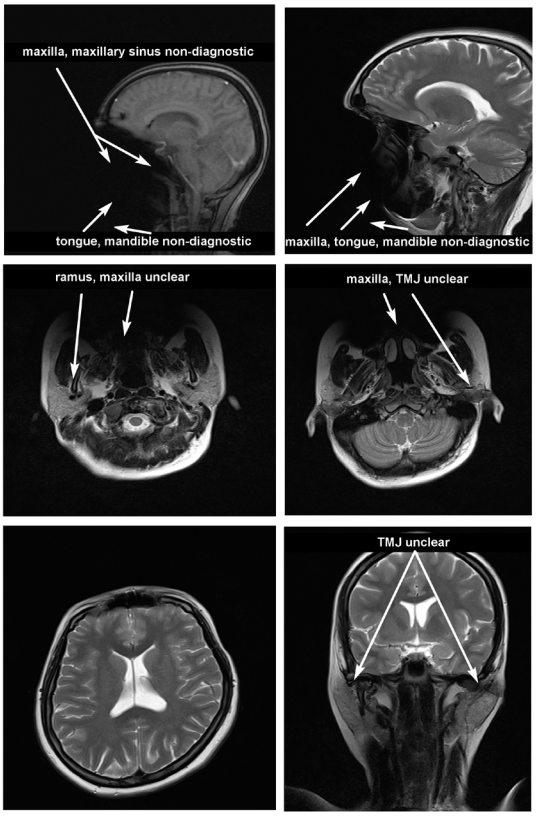

Fig. 1. Magnetic resonance imaging (MRI) scans of the head and neck region with 3M Unitek Gemini stainless steel buccal/labial brackets

TMJ – temporomandibular joint.

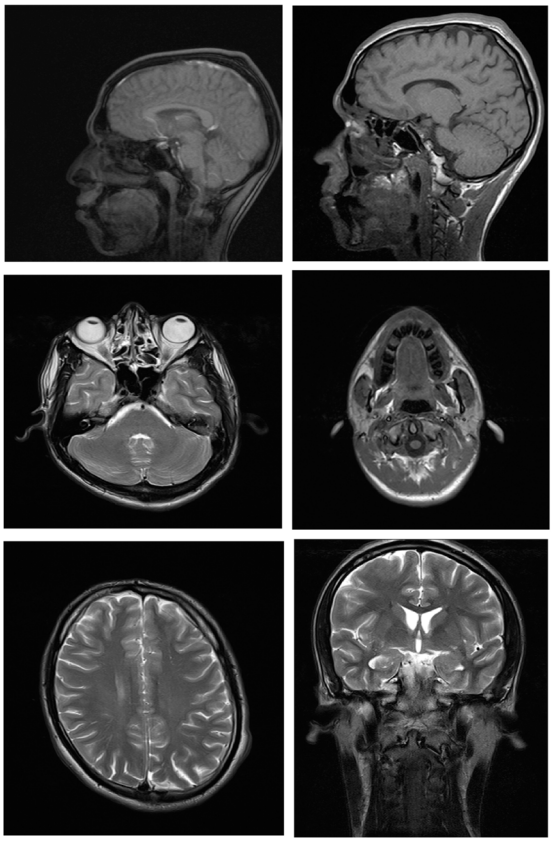

Fig. 3. Magnetic resonance imaging (MRI) scans of the head and neck region with 3M Unitek Clarity SL ceramic self-ligating brackets with metal slots

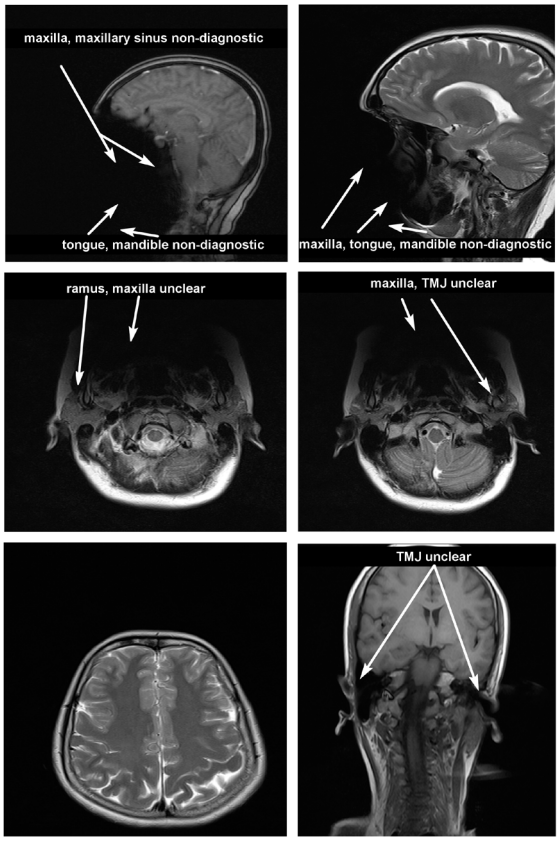

Fig. 2. Magnetic resonance imaging (MRI) scans of the head and neck region with Classic Orthodontics Invisible 7G stainless steel lingual brackets

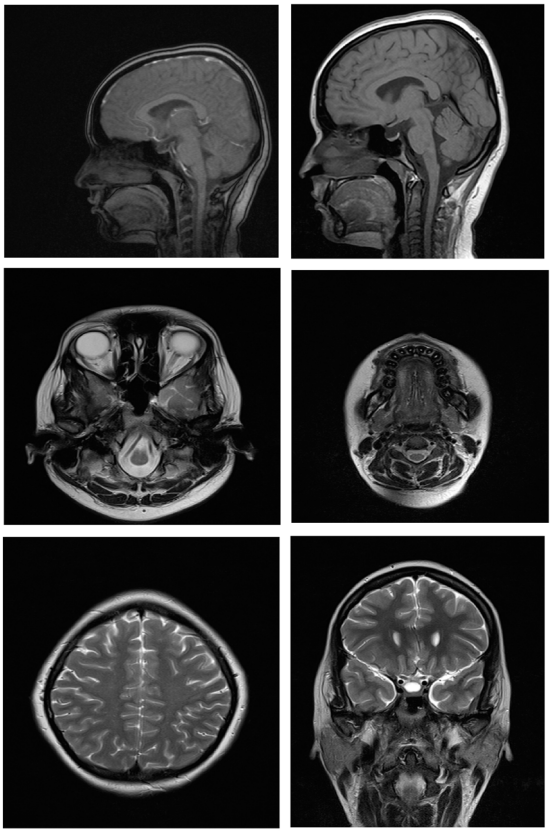

Fig. 4. Magnetic resonance imaging (MRI) scans of the head and neck region with Ormco Inspire ICE ceramic brackets

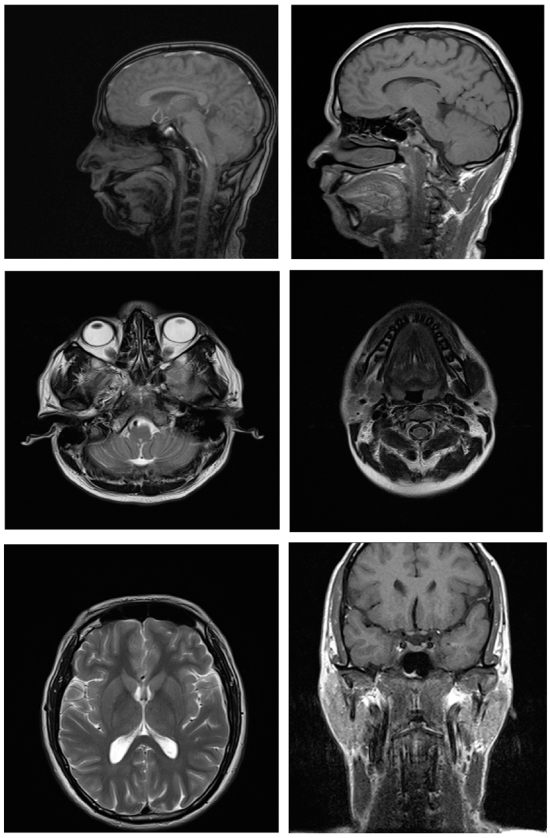

Fig. 5. Magnetic resonance imaging (MRI) scans of the head and neck region with JJ Orthodontics Ora-plus polycarbonate brackets

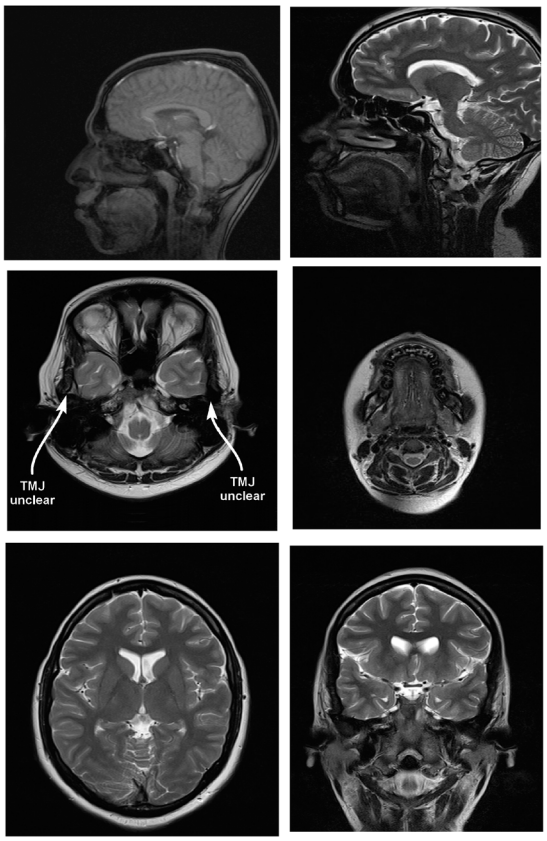

Fig. 6. Magnetic resonance imaging (MRI) scans of the head and neck region with an Ortho-Direct titanium retainer in the mandibular arch

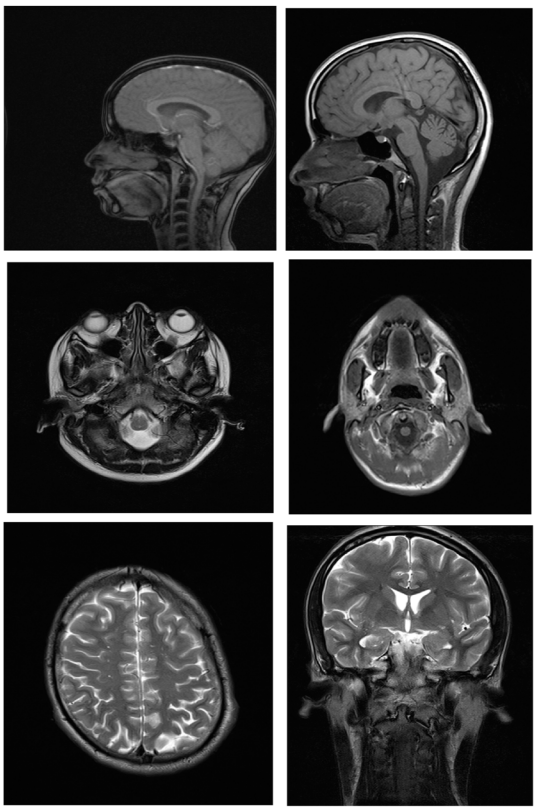

Fig. 7. Magnetic resonance imaging (MRI) scans of the head and neck region with a GC America everStick ORTHO fiber-reinforced composite retainer in the mandibular arch

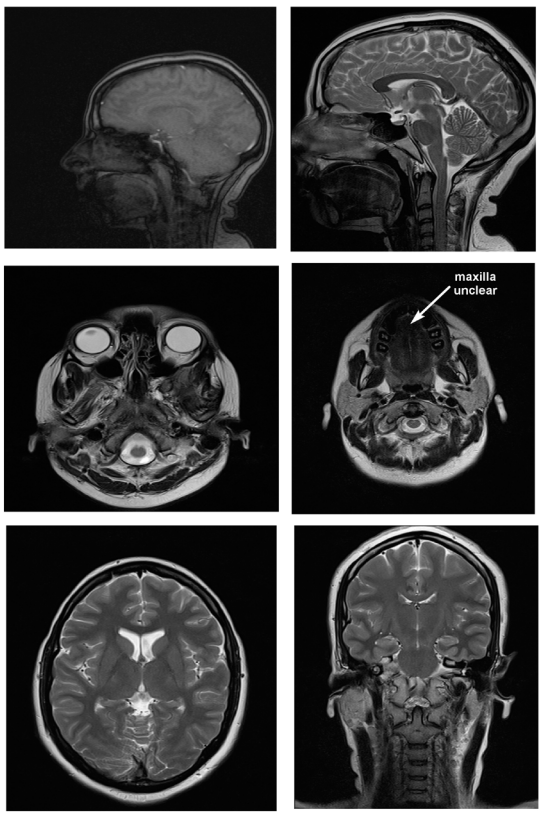

Fig. 8. Magnetic resonance imaging (MRI) scans of the head and neck region with multi-stranded stainless steel retainers in the maxillary and mandibular arches

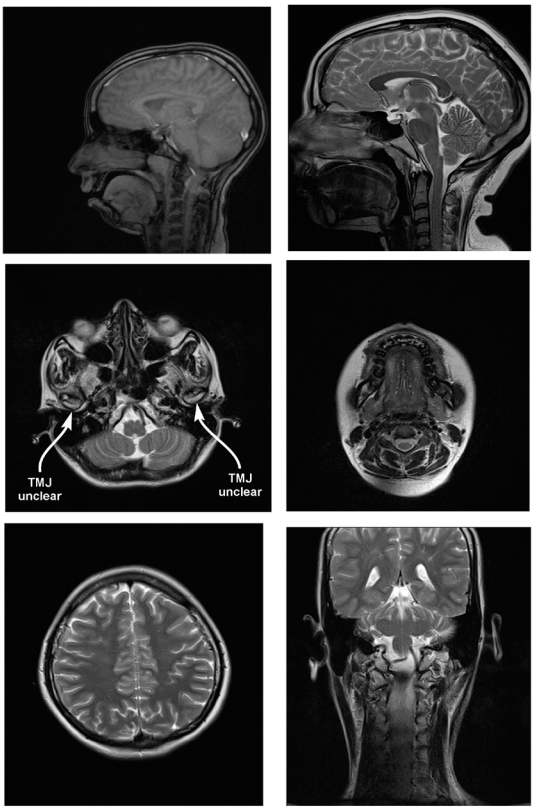

Fig. 9. Magnetic resonance imaging (MRI) scans of the head and neck region with a combination of a stainless steel retainer in the maxillary arch and a titanium retainer in the mandibular arch

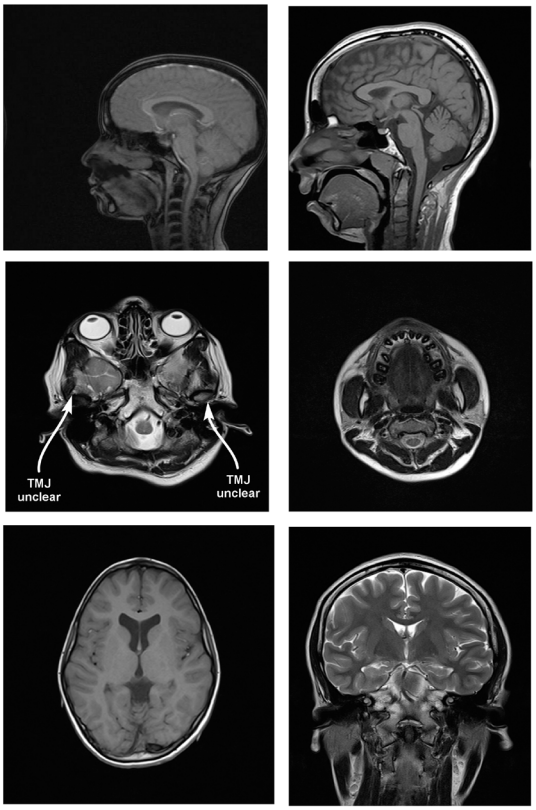

Fig. 10. Magnetic resonance imaging (MRI) scans of the head and neck region with a combination of a stainless steel retainer in the maxillary arch and a fiber-reinforced composite retainer in the mandibular arch

Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics for Windows software, v. 19.0 (IBM Corp., Armonk, USA). Non-parametric tests were used for the study. Differences between the materials and the anatomic sites with regard to the distortion rating scale were analyzed for statistical significance with the paired Wilcoxon signed-rank test. A p-value <0.05 was considered a statistically significant difference. The interrater agreement for each pair of evaluators was assessed for each material and each anatomic site with Cohen's kappa coefficient (κ).

Results

Statistically significant differences were found between the mean distortion scores given to stainless steel brackets (both buccal/labial and lingual) and to other bracket and retainer materials as well as controls ($p < 0.05$) (Table 3).

Table 3. Individual pairwise comparisons between various brackets and various retainers vs. stainless steel brackets (paired Wilcoxon signed-rank test)

Comparison	*Z*-value	*p*-value
Ceramic SL brackets with metals slots vs. SS brackets	–2.333	0.020*
Ceramic brackets vs. SS brackets	–2.264	0.024*
Polycarbonate brackets vs. SS brackets	–2.333	0.020*
Ti retainer (L) vs. SS brackets	–2.333	0.020*
FRC retainer (L) vs. SS brackets	–2.264	0.024*
SS retainers (U/L) vs. SS brackets	–2.333	0.020*
SS retainer (U)/Ti retainer (L) vs. SS brackets	–2.333	0.020*
SS retainer (U)/FRC retainer (L) vs. SS brackets	–2.333	0.020*

SL – self-ligating; SS – stainless steel; Ti – titanium; FRC – fiber-reinforced composite; U – upper arch; L – lower arch; * statistically significant.

Table 4 presents the mean distortion scores for particular bracket and retainer materials based on the scores obtained for each subject. Each subject received a mean distortion score above 3 for stainless steel buccal and lingual brackets, indicating that the images were non-diagnostic. Each subject received a mean distortion score below 3 for other bracket and retainer materials, which indicates that these MR images were diagnostic.

Table 4. Descriptive statistics regarding the distortion scores for all bracket and retainer materials

Material	*N*	*M*	*SD*	min	max
SS buccal/labial brackets	6	3.67	0.52	3	4
SS lingual brackets	6	3.67	0.52	3	4
Ceramic SL brackets with metal slots	6	1.67	0.52	1	2
Ceramic brackets	6	1.00	0.00	1	1
Polycarbonate brackets	6	1.00	0.00	1	1
Ti retainer (L)	6	2.00	0.00	2	2
FRC retainer (L)	6	1.00	0.00	1	1
SS retainers (U/L)	6	2.00	0.00	2	2
SS retainer (U)/ Ti retainer (L)	6	2.00	0.00	2	2
SS retainer (U)/FRC retainer (U)	6	2.00	0.00	2	2

N – number of evaluators; *M* – mean; *SD* – standard deviation; min – minimum; max – maximum.

Table 5 details the mean distortion scores obtained for different types of brackets and retainers according to the anatomic sites for all subjects. All sequences and all radiologists were considered when determining these mean values. Except for stainless steel buccal/labial and lingual brackets, other materials had an average distortion score below 3 with regard to various anatomic sites, indicating that these MR images were diagnostic. For stainless steel buccal/labial and lingual brackets, the mean distortion scores were above 3 consistently for each anatomic site except for the posterior cerebral fossa (a score of 1.16). This indicates that these MR images were non-diagnostic for all areas except for the posterior cerebral fossa.

Table 6 presents the results of the interrater reliability test with the use of Cohen's kappa coefficient (κ), concerning the assessments of the 6 radiologists. Excellent consistency between the examiners was obtained with a 98% (0.986) statistical value.

Table 5. Descriptive statistics regarding the distortion scores for all bracket and retainer materials according to various anatomic sites

Material	TMJ	Tongue	Maxilla	Mandible	Maxillary sinus	Posterior cerebral fossa
SS buccal/labial brackets	4.00 ±1.15	4.00 ±1.15	4.00 ±1.15	4.00 ±1.15	4.00 ±1.15	1.16 ±0.86
SS lingual brackets	4.00 ±1.15	4.00 ±1.15	4.00 ±1.15	4.00 ±1.15	4.00 ±1.15	1.16 ±0.86
Ceramic SL brackets with metal slots	1.60 ±0.24	1.60 ±0.24	1.60 ±0.24	1.60 ±0.24	1.60 ±0.24	1.00 ±0.00
Ceramic brackets	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00
Polycarbonate brackets	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00
Ti retainer (L)	2.16 ±0.42	2.00 ±0.42	2.00 ±0.42	2.00 ±0.42	2.00 ±0.42	1.00 ±0.42
FRC retainer (L)	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00	1.00 ±0.00
SS retainers (U/L)	2.16 ±0.42	2.00 ±0.42	2.00 ±0.42	2.00 ±0.42	2.00 ±0.42	1.00 ±0.42
SS retainer (U)/ Ti retainer (L)	2.16 ±0.65	2.00 ±0.65	2.00 ±0.65	2.00 ±0.65	2.00 ±0.65	1.00 ±0.65
SS retainer (U)/FRC retainer (L)	2.00 ±0.45	2.00 ±0.45	2.00 ±0.45	2.00 ±0.45	2.00 ±0.45	1.00 ±0.45

Data presented as *M* ±*SD*.

Table 6. Results of the interrater reliability test

Interrater reliability test	*ICC*	95% *CI*		*F* test with the true value 0		
		lower bound	upper bound	value	df1	df2
Single measures	0.922	0.829	0.977	81.250	9	45
Average measures	0.986	0.967	0.996	81.250	9	45

ICC – intraclass correlation coefficient with Cohen's kappa coefficient (κ); *CI* – confidence interval; df – degree of freedom.

Discussion

The increasing availability of MRI machines and ever-improving MRI technology has led to cranial MRI diagnostic techniques being used more frequently in medicine and other health-related fields. For example, over the past 10–15 years, MRI has improved our understanding and management of multiple sclerosis and other demyelinating disorders.[18–20]

With an increase in orthodontic awareness and growth in the number of orthodontists around the world, the number of patients seeking orthodontic treatment has also increased. Hence, it is expected that some patients undergoing orthodontic treatment or having fixed retainers after the completion of their orthodontic treatment may require MRI for various reasons. Radiologists currently ask orthodontists to remove orthodontic appliances before cranial MRI, as they lack the knowledge on orthodontic materials and their possible influence on the diagnostic image quality of MRI scans.

Previous studies have determined that some metals used in dentistry cause cranial MR image distortion. Lissac et al. discovered that many dental materials caused significant distortion of cranial MR images, and therefore should not be used for fixed dental work.[21] Masumi et al. supported this observation by concluding that dental materials that cause MR image distortion adversely affected the diagnosis of abnormalities in the craniocervical region.[22] Sadowsky et al. concluded that the areas closest to the problem material (metal) were the most distorted, while the anatomic sites farthest from the problem material (metal) were the least distorted.[12]

In the present study, stainless steel buccal/labial and lingual orthodontic brackets caused more distortion of cranial MR images and received higher distortion scores for all anatomic sites than other orthodontic bracket and retainer materials. The mean distortion score assigned by the radiologists to stainless steel appliances was 3.67. According to the anatomic landmarks, it was 4 by the same radiologists for TMJ, the tongue, the maxilla, the mandible, and the maxillary sinus, which means maximum distortion of the MR images. However, it was only 1.16 for the posterior cerebral fossa. This indicates that stainless steel buccal/labial and lingual brackets caused distortion that was most severe in the anatomic sites adjacent to them. The mean score of 1.16 for the posterior cerebral fossa means that there was almost no distortion, which shows a pattern of reduced artifacts with an increasing distance of the anatomic site from the brackets.

Since stainless steel buccal/labial and lingual brackets consistently had higher distortion scores, the MR images were considered non-diagnostic. The results of this study are similar to those obtained by Patel et al.,[8] Elison et al.,[17] Razdan and Rani,[23] Beau et al.,[24] Zhylich et al.,[25] and Cassetta et al.[26] However, they are contradictory to the research done by Sadowsky et al., since an older version of an MRI machine was used in their study.[12]

Ceramic self-ligating brackets with metal slots had a mean distortion score of 1.67 and mean distortion scores of 1.6 at all 6 anatomic sites except for the posterior cerebral fossa (1.0). This indicates minimal distortion for all ROI. Hence, the MR images taken with self-ligating brackets were diagnostic. A study conducted by Asano et al., in which 3 types of brackets (titanium, ceramic and ceramic with metal slots) were used in a single patient, concluded that the MR images showed little distortion and were interpretable.[27] Since all 3 types of brackets were used in a single patient, one cannot distinguish their individual effects on the MRI scans.[27]

The mean distortion scores for ceramic and polycarbonate brackets were 1.00, and the mean distortion scores at all 6 anatomic sites were also 1.00, which implies no distortion for any ROI; hence, the MR images with ceramic and polycarbonate brackets were considered diagnostic.

In recent years, many new fixed retainers have entered the market. Some of them include titanium retainers, fiber-reinforced composite retainers and braided titanium retainers. Retention is an important aspect in orthodontics. Many patients need to have fixed retainers for an extended period of time and may require MRI for various reasons during that time; hence, 3 types of fixed retainers and 2 combinations of them were evaluated for their influence on the diagnostic image quality of MRI scans. To date, there are no comprehensive MRI studies on these fixed retainers and combinations of these retainers in both arches.

Stainless steel retainers in both the maxillary and mandibular arch had a mean distortion score of 2.00. The mean distortion scores at the anatomic sites were 2.16 for TMJ, and 2.00 for the tongue, the maxilla, the mandible, and the maxillary sinus, with only the posterior cerebral fossa having a score of 1.00. This means that there was minimal or no distortion for all ROI. Hence, the MR images with stainless steel retainers in the maxillary and mandibular arches were considered diagnostic.

Titanium retainers in the mandibular arch alone had a mean distortion score of 2.00. The mean distortion scores at the anatomic sites were 2.16 for TMJ, 2.00 for the tongue, the maxilla, the mandible, and the maxillary sinus, and 1.00 for the posterior cerebral fossa, which indicates that there was minimal or no distortion for all ROI. Hence, the MR images with titanium retainers in the mandibular arch alone were considered diagnostic. The results of this study are similar to the findings reported in studies by Shalish et al.,[28] Beau et al.[24] and Zhylich et al.[25]

Fiber-reinforced composite retainers in the mandibular arch alone had a mean distortion score of 1.00 and mean distortion scores of 1.00 at all 6 anatomic sites, which means there was no distortion for any ROI. Hence, the MR images with fiber-reinforced composite

retainers in the mandibular arch only were considered diagnostic. There are no studies reporting on this type of retainers.

Sometimes, a combination of different fixed retainers is placed in the maxillary and mandibular arches based on the orthodontist's choice, the availability of retainers or the patient's preference. Hence, combinations of fixed retainers placed in the maxillary and mandibular arches were also examined in this study, as they have not been studied until now.

The combination of a stainless steel retainer in the maxillary arch and a fiber-reinforced retainer in the mandibular arch had a mean distortion score of 2.00 and mean distortion scores of 2.00 for TMJ, the tongue, the maxilla, the mandible, and the maxillary sinus; the posterior cerebral fossa had a score of 1.00. Therefore, the MR images with this combination caused minimal or no distortion, and were considered diagnostic by the radiologists.

The combination of a stainless steel retainer in the maxillary arch and a titanium retainer in the mandibular arch received similar scores to the previous combination. Therefore, the MR images with a stainless steel retainer in the maxillary arch and a titanium retainer in the mandibular arch were considered diagnostic.

Conclusions

Stainless steel buccal/labial and lingual brackets caused maximum distortion of the MR images, rendering them non-diagnostic; hence, they should be removed before MRI. Ceramic and polycarbonate brackets as well as fiber-reinforced composite retainers did not distort the images, so they need not be removed before MRI. Ceramic self-ligating brackets with metal slots, titanium retainers, stainless steel retainers, and combinations of these fixed retainers caused minimal distortion, but the MR images were still diagnostic. Hence, these types of materials may not need to be removed prior to MRI.

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Correlation between the upper airway volume and the hyoid bone position, palatal depth, nasal septum deviation, and concha bullosa in different types of malocclusion: A retrospective cone-beam computed tomography study

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Abstract

Background. The upper airway volume is among the factors that affect orthodontic treatment plans. Cone-beam computed tomography (CBCT), as an accurate diagnostic modality, can help assess anatomical structures associated with the upper airway volume.

Objectives. This study aimed to use CBCT to determine if there are differences in the upper airway volume between different sagittal and vertical skeletal patterns, considering the hyoid bone position, palatal depth, nasal septum deviation (NSD), and concha bullosa.

Material and methods. From among 105 initial CBCT samples retrieved from the archive of a private radiology clinic in Tehran, Iran, 90 CBCT scans of 27 males and 63 females aged 17–65 years were considered in the study according to the inclusion criteria. The upper airway volume was assessed with regard to Angle's classification (using the A point–nasion–B point angle (ANB)), the vertical skeletal dimension (using the sella–nasion plane to mandibular plane angle (SN–MP)), the hyoid bone position, palatal depth, NSD, and concha bullosa, using CBCT and the NNT® software. The one-way analysis of variance (ANOVA), Levene's test and the *t* test were used to analyze the data with the SPSS Statistics for Windows software, v. 17.0.

Results. The upper airway volume was significantly smaller in long-face cases ($p = 0.037$). There was no significant correlation between the upper airway volume and Angle's classification, the hyoid bone position, palatal depth, NSD, and concha bullosa.

Conclusions. The vertical skeletal dimension was the only parameter that was related to the upper airway volume. The results of this study can be considered while preparing orthodontic treatment plans.

Keywords: upper respiratory tract, pharynx, three-dimensional assessment, jaw relationship

Introduction

The relationship between the airway volume and different types of malocclusion has been researched for many years. The upper airway volume is very important in orthodontics, as it is related to craniofacial growth and development. It may be affected by different positions of the jaws and may determine various treatment plans. Trying to identify and manage the confounding factors with regard to the upper airway volume can be helpful in orthodontic treatment. The upper airway volume may be affected by sagittal (class I, II and III) and vertical (short, normal and long face) dentoskeletal malocclusion as well as facial morphology.[1,2] Also, conditions such as functional anterior shifting,[3] the head posture,[4] maxillary protraction,[5] palatal depth, the hyoid bone position,[6] nasal septum deviation (NSD), and concha bullosa have effects on the airway volume.[7]

The relationship between various types of dentoskeletal malocclusion and the upper airway volume is differently described in different studies.[1,2,8] Sahoo et al. evaluated the hyoid bone position in skeletal class II malocclusion after the advancement of the mandible and realized that the hyoid bone moves forward as the mandible is advanced.[9] Although some studies have evaluated the relationship between maxillary expansion and the upper airway volume,[10] there are few studies on the relationship between palatal depth and the airway volume.

The airway volume has been measured with various imaging techniques, including computed tomography (CT), cone-beam computed tomography (CBCT), cephalometry, fluoroscopy, nasopharyngoscopy, and magnetic resonance imaging (MRI).[11] Most of the previous studies had limitations, because they evaluated the upper airway volume based on patients' lateral cephalograms. A three-dimensional (3D) CBCT system provides more reliable landmark identification of anatomical structures than two-dimensional (2D) cephalometry. Cone-beam computed tomography allows the exact measurement of the airway space and identifies different types of malocclusion in orthodontics. A lower radiation dose, lower costs, shorter scanning time, and overall accuracy have made the CBCT technology a preferred method to evaluate the airway volume.[12]

The aim of this study was to use CBCT to evaluate the upper airway volume in different types of malocclusion with regard to special criteria, such as the hyoid bone position, palatal depth, NSD, and concha bullosa.

Material and methods

This retrospective cross-sectional study was performed using the CBCT images of 105 patients, randomly selected from the archive of a private radiology clinic in Tehran, Iran. Ninety CBCT scans of 27 males and 63 females aged 17–65 years were included in the study. The inclusion criteria were: complete medical history records; no dentofacial syndromes; and no history of orthodontic treatment, orthopedic maxillary expansion, orthognathic surgery, tonsillectomy, or adenoidectomy. The CBCT scans were excluded if the airway was not clear, the hyoid bone position as well as the nasion (N) and sella (S) points were not obvious, or if there were artifacts.

The CBCT scans and the patients' medical history forms were gathered. All CBCT images were obtained using a NewTom® VG machine (NewTom, Imola, Italy) with the following settings: 2 mA; 110 kVp; 10 s; and a field of view (FoV) of 18 cm × 18 cm. A resolution of 1,024 × 31,024 pixels and 12 bits per pixel (4,096 gray scale) was applied. The images were taken with the teeth in maximum intercuspation, a natural head position, and at the end of the exhalation period, when the patient was not swallowing. Lateral cephalograms were obtained for all patients based on the CBCT data.

According to Angle's classification using the A point–nasion–B point angle (ANB), the samples were divided into 3 groups: class I ($-0.5° < ANB < 4.5°$) ($n = 42$); class II ($ANB \geq 4.5°$) ($n = 34$); and class III ($ANB \leq -0.5$) ($n = 14$).[13] The sella–nasion plane to mandibular plane angle (SN–MP) was used to categorize different vertical growth patterns (low angle: <26°; normal angle: 26–38°; and high angle: >38°).[14]

The distance from the most prominent point of the hyoid bone to menton (Me) was measured to evaluate the hyoid bone position sagittally. Also, the distance between the most prominent point of the hyoid bone and the mandibular plane, i.e., the gonion–gnathion line (Go–Gn), was considered as the hyoid bone position in the vertical dimension. The parasagittal view was used for these measurements (Fig. 1).

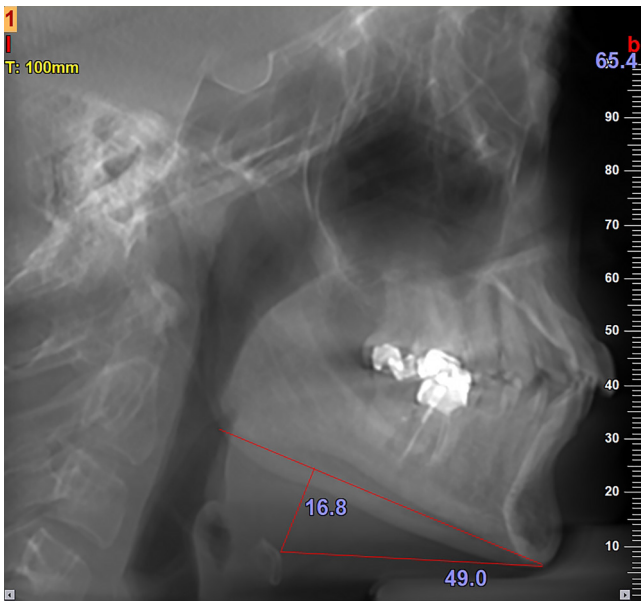

Fig. 1. Evaluation of the hyoid bone position based on menton (Me) and the mandibular plane (Go–Gn)

To assess palatal depth, a transverse line was drawn between the mesiobuccal cusp tips of maxillary first molars in the axial view. Then, the distance between this transverse line and the palatal bone in the midline of the coronal view was considered to be palatal depth (Fig. 2).

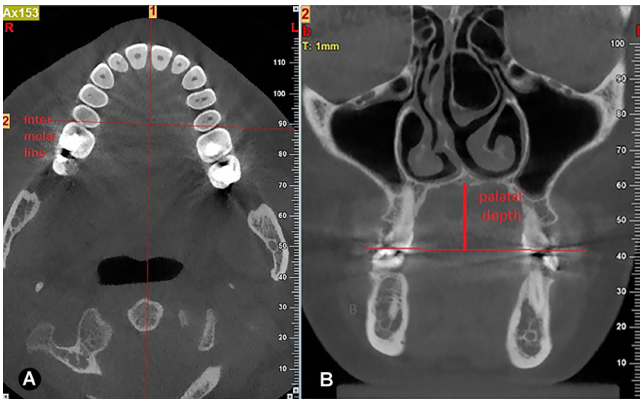

Fig. 2. Evaluation of palatal depth
A – axial view; B – coronal view.

All measurements were obtained by an experienced orthodontist via the export of the CBCT data in the DICOM format and its import into the NNT® viewer, v. 2.21 (NewTom).

The oropharyngeal airway was circumscribed by the palatal plane, i.e., the anterior nasal spine–posterior nasal spine line (ANS–PNS), reaching to the posterior wall of the pharynx, and a line parallel to the palatal plane, extending to the most antero-inferior point of the second cervical vertebra (C2 dens).

The inferior limit of the nasopharynx was ANS–PNS and the superior limit was the last slice before the posterior wall of the pharynx, fusing with the nasal septum. It was observed in the axial view first, and then projected to the sagittal view (Fig. 3). The upper airway volume was calculated with the NNT software (Fig. 4).

Any deviation greater than 4 mm in the midpoint of the nasal septum with regard to the line of symmetry was defined as NSD in the coronal view. Concha bullosa was considered to be the presence of pneumatization of any size within the inferior, middle or superior conchae.[15]

After 2 weeks, the scans of 20 patients were randomly reassessed to determine the reliability of the measurements. Applying Dahlberg's formula (Equation 1) to the 1st and 2nd angular and linear measurements revealed no considerable differences between them.

$$EM = \sqrt{\frac{\sum d^2}{2n}} \quad (1)$$

where:
EM – error of measurement;
d – difference between the 2 recordings for the individual;
n – number of double recordings.

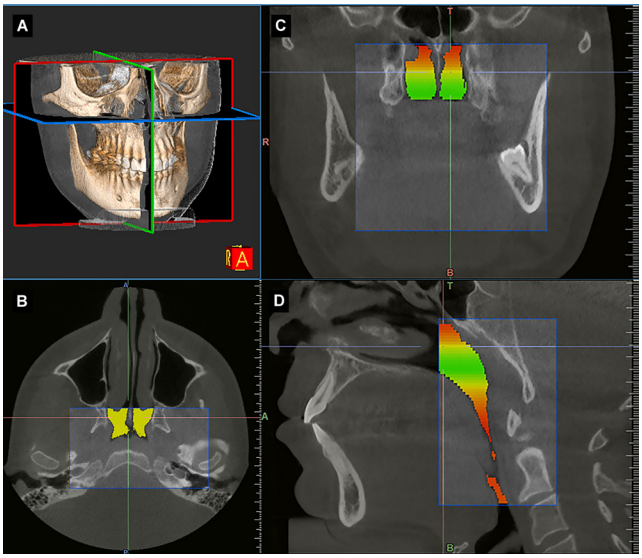

Fig. 3. Measurement of the upper airway volume
A – axial, coronal and sagittal planes; B – the last slice before the posterior wall of the pharynx, fusing with the nasal septum in the axial view; C – coronal view; D – parasagittal view, extending to the most antero-inferior point of the second cervical vertebra (C2 dens)

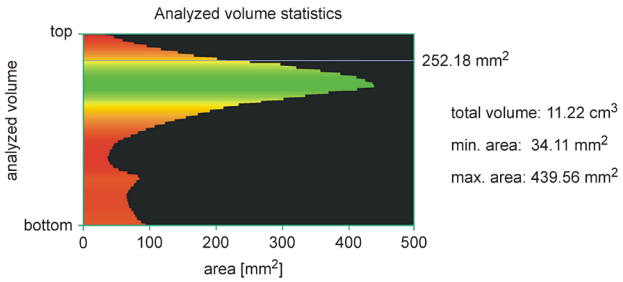

Fig. 4. Calculating the upper airway volume with the NNT software

Statistical analysis

The one-way analysis of variance (ANOVA) was used to assess differences in the total upper airway volume with regard to age and gender among different skeletal classification groups. Levene's test was used to analyze the equality of variances and the *t* test for the equality of means. The SPSS Statistics for Windows software, v. 17.0 (SPSS Inc., Chicago, USA) was used for the statistical analysis. A p-value <0.05 was considered statistically significant.

Results

From among 105 initial CBCT samples, 90 CBCT scans of 27 males and 63 females aged 17–65 years were analyzed in the study. According to the multiple linear regression analysis, the upper airway volume was significantly different for various vertical skeletal dimensions. A smaller airway volume was detected in long-face cases (p = 0.037).

The upper airway volume was significantly increased in older subjects ($p = 0.042$) and it was significantly increased in females ($p = 0.036$). There was no significant relationship between the upper airway volume and malocclusion in terms of Angle's classification ($p = 0.541$). There was no correlation between the upper airway volume and the hyoid–menton distance (H–Me), the hyoid–mandibular plane distance (H–MP), palatal depth, and concha bullosa. Pearson's correlation coefficients (r) and p-values for particular parameters with regard to the upper airway volume are presented in Table 1.

According to the t test analysis, there was no statistically significant relationship between NSD and the following parameters: H–Me; H–MP; palatal depth; and the upper airway volume ($p > 0.05$).

There was a statistically significant negative relationship between H–Me and the vertical dimension. The distance H–Me was greater in short-face cases ($p = 0.002$). The distance H–Me was not statistically significantly different with regard to various types of malocclusion according to Angle's classification, age and gender ($p > 0.05$).

There was a statistically significant relationship between H–MP and Angle's classification (class III > class I > class II) ($p = 0.018$). The distance H–MP was statistically significantly greater in males ($p = 0.008$) and in patients with concha bullosa ($p = 0.001$).

Palatal depth was reported to be statistically significantly greater in males ($p < 0.05$).

The mean (M) and standard deviation (SD) values for particular airway parameters with regard to Angle's classification and vertical growth patterns are presented in Table 2.

Table 1. Correlations between the upper airway volume and the parameters studied

Parameter	*r*	*p*-value†
Angle's classification	0.063	0.541
Vertical growth pattern	–0.214	0.037*
Age	0.210	0.042*
Male gender	–0.215	0.036*
H–Me	0.136	0.201
H–MP	–0.117	0.274
Palatal depth	–0.054	0.611

r – Pearson's correlation coefficient; H–Me – hyoid–menton distance; H–MP – hyoid–mandibular plane distance; † – ANOVA; * statistically significant.

Discussion

The upper airway volume is vital in orthodontics, as it is related to craniofacial growth and development.[16] The upper airway volume is associated with different types of malocclusion and various structures, such as NSD, concha bullosa, and the hyoid and palatal bones. Therefore, the aim of this study was to use CBCT to evaluate the upper airway volume in different types of malocclusion and consider the associated structures.

This study found that there was a noticeable relationship between the upper airway volume and the vertical craniofacial dimension. The upper airway volume was increased in short-face cases. Also, it was increased in older subjects. The upper airway volume was greater in females in comparison with males. Angle's classification, the hyoid bone position, palatal depth, NSD, and concha bullosa were not statistically significantly related to the upper airway volume.

Lateral cephalometry provides limited evaluation of the airway, as it is a 2D sagittal projection.[17] In recent years, CBCT has improved the accuracy of the analysis of the airway space and different types of malocclusion in orthodontics.[18] Therefore, CBCT was used for all measurements in this study.

Some researchers claim that there is a correlation between a smaller airway volume and class II malocclusion.[19] On the other hand, Shokri et al. observed a greater airway volume in skeletal class III cases, but the difference between class I and class II was not statistically significant.[2] In the present study, however, there was no significant relationship between different types of malocclusion according to Angle's classification and the airway volume. Differences in the values of the airway volume with respect to Angle's classification may be attributed to the chosen sensitivity values and the ANB ranges, which were different in different studies.

In contrast to the present study, some researchers have shown that the airway volume is not statistically significantly different with respect to the vertical craniofacial dimension.[1] Conversely, in a study similar to the present one, Alhammadi et al. determined that the airway volume was decreased in long-face cases.[20] The contradicting results might be due to the different age ranges of the study groups.

The hyoid bone plays an important role in the maintenance of the upper airway space and its position changes according to the different positions of the mandible.

Table 2. Comparison of the airway parameters with regard to Angle's classification and vertical growth patterns

Airway parameter	Angle's classification			Vertical growth pattern		
	class I	class II	class III	short face	normal face	long face
Upper airway volume	17.523 ±6.646	21.730 ±5.725	20.013 ±6.323	21.936 ±6.146	18.988 ±6.663	18.249 ±6.222
H–Me	46.291 ±4.963	49.679 ±7.399	47.983 ±5.142	50.532 ±7.101	47.842 ±4.987	45.655 ±4.389
H–MP	14.674 ±5.190	11.264 ±4.564	14.835 ±4.438	13.737 ±4.953	13.734 ±4.770	15.015 ±5.158
Palatal depth	21.006 ±3.533	19.614 ±2.600	19.421 ±2.571	19.216 ±2.850	20.171 ±3.163	20.391 ±2.996

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

Based on our measurements, there was a highly significant relationship between H–MP and Angle's classification (class III > class I > class II). There was no statistically significant relationship between the hyoid bone position and the upper airway volume. Similarly, in a long-term study, the hyoid bone moved significantly forward after mandibular advancement operations on class II cases, but the airway volume remained almost unchanged.[21] In contrast, in a study of class III patients, the hyoid position did not change after mandibular setback surgery in the long term, but the nasopharynx volume increased significantly.[22] The conflicting results of these studies may be due to the different landmarks in the assessment of the anatomical position of the hyoid bone. In the present study, in order to increase the accuracy of measurements, the hyoid bone position was determined based on Me (as the sagittal position) and MP (as the vertical position). In other studies, the distance between the hyoid bone and retrognathion (RGn) (the most prominent point of the posterior border of the mandibular symphysis) was considered to determine the hyoid bone position.[23] Also, the conflicting results may be due to the chosen radiological modality. Some researchers have stated that the hyoid triangle method was applicable to lateral cephalometry, but not to 3D CBCT; therefore, CBCT showed a lesser correlation between the hyoid bone position and the airway volume.[6] Furthermore, the tone of the hyoid muscular suspension apparatus is different in the upright and supine positions. In the present study, the examination was performed with a NewTom VG CBCT unit in the upright position. Therefore, the results of various studies may differ due to the different positions of patients during examination.

The relationship between palatal depth and the upper airway volume was not noticeable in the present study. Similarly, many investigations have shown that there is no statistically significant relationship between these two items.[24] This is in contrast with a recent study showing that a smaller palatal depth was correlated with the airway obstruction in children.[25] The cause of such conflicting results may be the different age ranges of patients.

Since the nasal septum is a relevant structure in the airway, we evaluated the relationship between NSD and the upper airway volume. The present assessment showed no correlation between NSD and the upper airway volume. Wanzeler et al. reported that NSD influenced the oropharynx volume.[7] They showed that the oropharynx volume was increased in NSD patients, although the presence of NSD was not associated with facial types.[7] The section of the scan in which NSD was evaluated is the probable reason for the differences between the studies.

Concha bullosa is a common anatomical problem that may accompany NSD. The present study did not find any statistically significant relationship between concha bullosa and the upper airway volume. This is similar to the research conducted by Balikci et al.[15] The authors of the present study predicted that the pneumatization of the conchae might decrease the nasopharyngeal space volume; however, the results of the study did not support this assumption.

The tongue dimension as a possible factor influencing the upper airway volume cannot be assessed accurately by means of CBCT. Thus, further studies are recommended with the use of other modalities, such as MRI.

Conclusions

In conclusion, the upper airway volume was greater in short-face subjects, and especially in older females. There was no correlation between the upper airway volume and the anatomical and morphological variations, such as malocclusion according to Angle's classification, the hyoid bone position and palatal depth.

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Micro-computed tomography assessment of dentinal microcracks after the preparation of curved root canals with rotary and reciprocal systems

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Abstract

Background. Root canal preparation with nickel-titanium (NiTi) instruments may lead to the formation of microcracks in the root canal wall. Vertical root fractures may initiate from dentinal cracks, and eventually necessitate tooth extraction.

Objectives. This study aimed to assess the effect of the instrumentation of curved root canals of mandibular molars with the 2Shape (2S) sequential rotary, EdgeFile® X1 (EFX1) reciprocating and NeoNiTi (NN) rotational single-file systems on the formation of dentinal microcracks with the use of micro-computed tomography (micro-CT).

Material and methods. Thirty curved mandibular molar root canals were instrumented with the 2S, EFX1 and NN systems (10 in each group). The teeth underwent micro-CT before and after instrumentation. Next, the pre-instrumentation and post-instrumentation cross-sectional images were evaluated and compared for the detection of dentinal microcracks. The number of microcracks in each group was calculated and reported as percentage. The data was analyzed using the McNemar's test with the IBM SPSS Statistics for Windows software, v. 25.0 ($\alpha = 0.05$).

Results. Out of the 29,280 cross-sectional images evaluated in this study, 11.5% showed dentinal microcracks ($n = 3,362$). On the post-instrumentation images, the frequency percentage of microcracks was 12.0% ($n = 585$) in the 2S group, 8.8% ($n = 402$) in the EFX1 group and 13.3% ($n = 694$) in the NN group. All of the microcracks detected on the post-instrumentation images were also present on the pre-instrumentation images and no new microcracks were formed after root canal instrumentation with the aforementioned systems.

Conclusions. Root canal instrumentation with the 2S, EFX1 and NN systems did not result in the formation of new dentinal microcracks.

Keywords: root canal preparation, microcracks, dentinal defect, micro-computed tomography, curved root canal

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Introduction

The main goal of the chemomechanical preparation of the root canal system is to eliminate microorganisms, pulpal tissue and debris, and to shape the root canal in order to create adequate space for the root filling material.[1] Nickel-titanium (NiTi) rotary systems with improved properties are produced by several manufacturers to achieve these goals. NiTi systems have numerous advantages over stainless-steel hand files, such as a higher flexibility and a shorter working time. However, root canal preparation with these instruments may lead to the formation of microcracks in the root canal wall.[2,3] Microcracks may occur as a result of the distribution of lateral forces in root canal walls and root surface strain. They appear more frequently when high-taper instruments are used or in teeth with curved roots, as the part of the canal with the maximum curvature accumulates the maximum stress.[4] The importance of this topic consists in the fact that vertical root fractures may initiate from dentinal cracks, and eventually necessitate tooth extraction.[5]

2Shape (2S; Micro-Mega, Besançon, France) instruments with sequential rotary systems are manufactured by means of specific heat treatment (T-Wire), which is believed to provide higher flexibility and cyclic fatigue resistance, and preserve the elasticity of NiTi files.[6]

EdgeFile® X1 (EFX1; EdgeEndo, Albuquerque, USA) is a reciprocating system manufactured from the annealed heat-treated NiTi alloy, commercially known as FireWire™. Its manufacturer claims that it has a high torque strength, optimal flexibility and an increased cyclic fatigue resistance.[7]

NeoNiTi (NN; Neolix, Châtres-la-Forêt, France) is a single-file system with a full-rotational motion that benefits from the electric discharge machining (EDM) technology. Its manufacturer claims that it has optimal flexibility, a high fracture resistance, and a favorable shaping ability due to its unique properties, rectangular cross-section, efficient cutting blades, and built-in abrasive surface.[8]

Micro-computed tomography (micro-CT) is an imaging modality suggested for the assessment of dentin and its changes without damaging the tooth structure. Due to its high accuracy, it is commonly used for the assessment of dentinal microcrack formation following root canal instrumentation.[2,3,9,10]

This study aimed to assess the formation of new dentinal microcracks following the instrumentation of curved root canals of mandibular molars with the 2S, EFX1 and NN systems by using micro-CT.

Material and methods

The study protocol was approved by the Research Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.DRC.REC.1399.036).

Sample size calculation

The sample size was calculated to be 10 in each group (to assess the effect of instruments on dentin), assuming the effect size of 7.6 for the microcracks reported after root canal instrumentation in previous studies,[11,12] $\alpha = 0.05$, $\beta = 0.95$, and a study power of 90%, using the PASS 15 software (NCSS, Kaysville, USA).

Sample selection

A total of 250 mandibular first and second molars were evaluated for inclusion in this study. All roots were inspected under a stereomicroscope at ×12 magnification. Teeth with immature apices and external dentinal defects were excluded. Next, the teeth underwent high-resolution cone-beam computed tomography (CBCT) with the use of a NewTom® VGi scanner (NewTom, Imola, Italy) with the exposure parameters of 110 kVp, 9.5 mA, a voxel size of 0.1 mm, and a field of view (FoV) of 6 × 6 cm. The NNT® viewer, v. 8.0 (NewTom) was used to assess different sections in order to classify the root canal system and root canal curvature. Root canal curvature was measured according to Schneider's method.[13] In this method, the degree of root canal curvature is determined by measuring the angle formed between the longitudinal axis of the root canal and a line drawn from the initiation point of curvature to the apical foramen. Molar teeth with a Vertucci type IV mesial root, a mesiobuccal root canal curvature of 20–40° on sagittal and/or coronal sections, no calcification, no internal or external root resorption, and no history of previous endodontic treatment were enrolled. The mesiobuccal canals of the mesial roots of 30 mandibular molars that met the eligibility criteria were selected.[14] All teeth were stored in a 0.1% thymol solution during the course of the study.

The crowns and distal roots of all teeth were cut with a low-speed saw (IsoMet™ 4000; Buehler Ltd., Lake Bluff, USA) under water cooling, and all remaining roots were standardized to have a length of 12 ±1 mm from the apex.

To simulate the periodontal ligament, the root surfaces were wrapped with 1 layer of aluminum foil and mounted in tubes filled with an acrylic resin (Kulzer, Hanau, Germany). After setting, the aluminum foil wrap was removed from the root surfaces and the created space was filled with a silicon impression material (GC Corporation, Tokyo, Japan). The roots were immediately placed back in the block.[12]

Each root was randomly assigned to one of the 3 groups of 2S, EFX1 or NN ($n = 10$) for root canal instrumentation. All roots underwent micro-CT (LOTUS-inVivo; Behin Negareh Co., Tehran, Iran) prior to instrumentation[15] and 400–500 micro-CT transverse cross-sectional images were obtained for each root. The exposure parameters included an isotropic resolution of 31 μm, a voltage of 99 kV,

an amperage of 88 μA, and a frame exposure time of 2 s, with an aluminum filter with a thickness of 0.5 mm, a rotation of 360° and a rotation step of 0.3°.

Root canal preparation

The working length was determined by introducing a size 10 K-file (Mani Inc., Utsunomiya, Japan) into the root canal until its tip was visible at the apical foramen; 1 mm was subtracted from this length to determine the working length. Next, a glide path was created using a size 15 K-file (Mani Inc.). Instrumentation was performed by the same operator in all groups.

Each file was used for the instrumentation of 2 canals, using an endomotor (Silver; VDW, Munich, Germany) with the speed and torque set as recommended by the manufacturer.

In the 2S group, TS1 (25, 04) and TS2 (25, 06) files were sequentially introduced into the canal with a torque of 1.5 N·cm and a speed of 300 rpm with a pecking motion, and proceeded with an up-and-down motion until the file reached the working length.

In the EFX1 group, the EdgeFile X1 instrument (25, 06) was used with a reciprocating motion at the WaveOne setting until it reached the working length.

In the NN group, the NeoNiTi A1 instrument (25, 06) with a torque of 1.5 N·cm and a speed of 300 rpm was used with a circumferential brushing motion until reaching the working length.

In all systems, after 3 pecking motions, the file was removed from the canal and its flutes were cleaned with gauze. Next, the root canal was rinsed with 2 mL of 2.5% sodium hypochlorite.[2] After root canal instrumentation, the root canals underwent micro-CT again with the same exposure parameters as those used for pre-instrumentation scanning.

Assessment of dentinal microcracks

The reconstructed micro-CT images were transferred to a data viewer (RadiAnt DICOM Viewer 2020.2). The pre- and post-instrumentation micro-CT cross-sectional images (N = 29,280) were simultaneously evaluated. Two blinded observers (endodontists) with 4 years of clinical experience evaluated all sections twice at a 2-week interval (to assess intra-observer agreement) and recorded the microcracks. A microcrack was defined as any incomplete crack line initiating from the canal wall and extending into the dentin structure, but not reaching the external surface of the root. A complete crack was defined as a crack line initiating from the root canal wall and extending to the external surface of the root. A craze line was defined as any other line that did not reach the root surface or extended from the external surface of the root into the dentin, but did not reach the root canal wall.[11] In case of any disagreement between the observers, the images were inspected again until consensus was reached.

Statistical analysis

Cohen's kappa coefficient (κ) was calculated to assess inter- and intra-observer reliability. The number of dentinal cracks before and after instrumentation in each group was compared by means of McNemar's test using the IBM SPSS Statistics for Windows software , v. 25.0 (IBM Corp., Armonk, USA), at a level of significance of 0.05.

Results

The kappa coefficient was calculated to be 0.86, which indicated very good inter-observer agreement (reliability). The kappa coefficient for intra-observer agreement (reliability) was found to be 1 for both observers, indicating complete agreement.

Out of the 29,280 cross-sectional images evaluated in this study, 11.5% showed dentinal microcracks (n = 3,362). On the post-instrumentation images, the frequency percentage of microcracks was 12.0% (n = 585) in the 2S group, 8.8% (n = 402) in the EFX1 group and 13.3% (n = 694) in the NN group. All the microcracks detected on the post-instrumentation images were also present on the pre-instrumentation images and no new microcracks were formed after root canal instrumentation with the aforementioned systems (p = 1.00) (Table 1).

Table 1. Dentinal microcracks on the cross-sectional images of the roots before and after instrumentation

Scanning time	Dentinal microcracks								
	2S			EFX1			NN		
	presence of microcracks	absence of microcracks	total	presence of microcracks	absence of microcracks	total	presence of microcracks	absence of microcracks	total
Before instrumentation	585 (12.0)	4,295 (88.0)	4,880 (100)	402 (8.8)	4,149 (91.2)	4,551 (100)	694 (13.3)	4,515 (86.7)	5,209 (100)
After instrumentation	585 (12.0)	4,295 (88.0)	4,880 (100)	402 (8.8)	4,149 (91.2)	4,551 (100)	694 (13.3)	4,515 (86.7)	5,209 (100)
Total	1,170 (12.0)	8,590 (88.0)	9,760 (100)	804 (8.8)	8,298 (91.2)	9,102 (100)	1,388 (13.3)	9,030 (86.7)	10,418 (100)

Data presented as number (percentage) (n (%)). 2S – 2Shape; EFX1 – EdgeFile X1; NN – NeoNiTi.

Figures 1–4 show the cross-sectional images of the roots before and after instrumentation. Table 2 presents the total frequency percentage of microcracks on the cross-sectional images of the roots in all groups. Figure 5 shows the sequence of cross-sectional images of the same mesial root, demonstrating the development of microcracks.

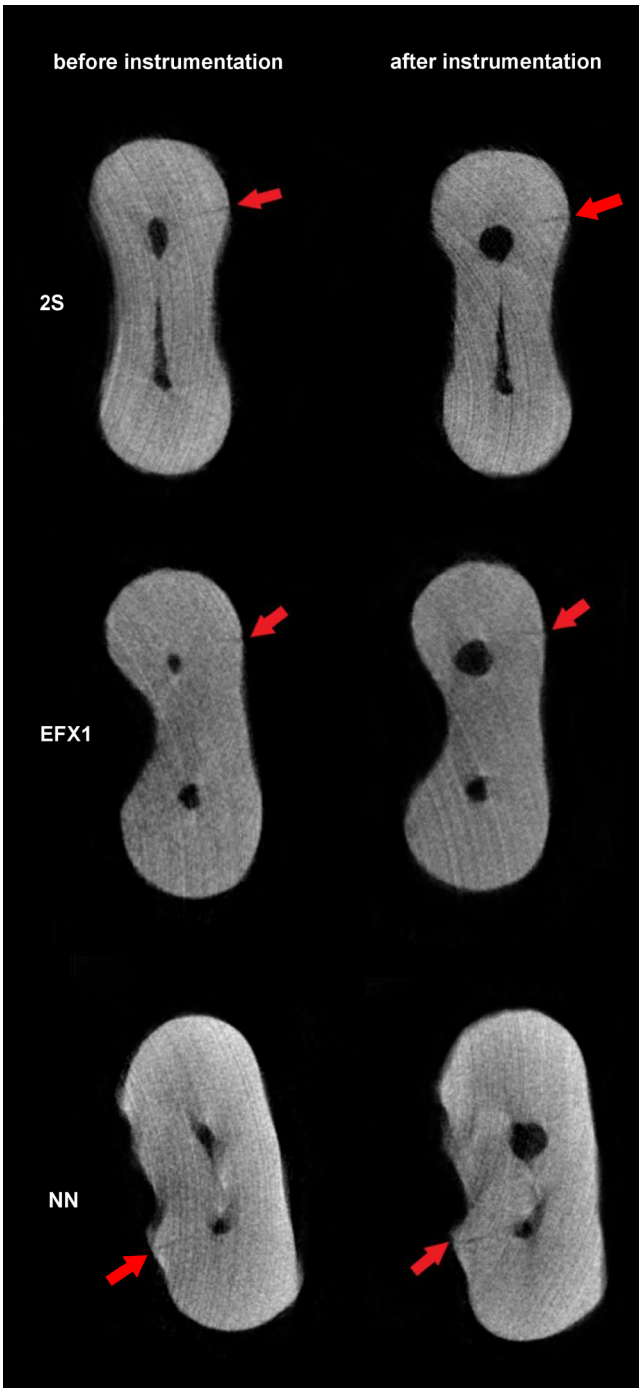

Fig. 1. Representative cross-sectional images of the mesial roots of mandibular molars showing the presence of cracks (arrows) before and after the preparation of the mesiobuccal canals with the 2Shape (2S), EdgeFile X1 (EFX1) and NeoNiTi (NN) systems

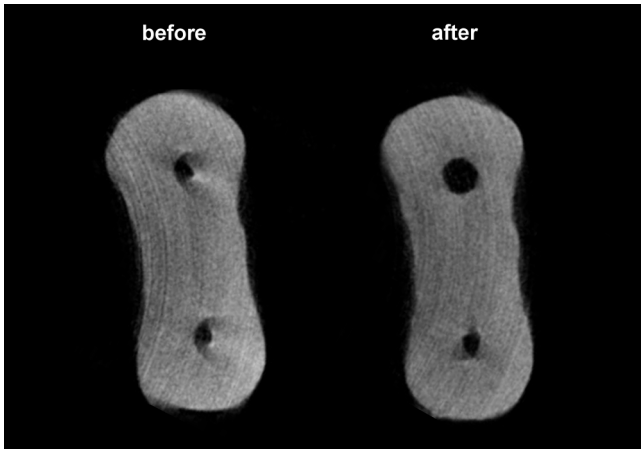

Fig. 2. Representative cross-sectional images of the roots, showing the absence of cracks before and after instrumentation

Table 2. Total frequency of microcracks on the cross-sectional images of the roots in all 3 study groups

Scanning time	Presence of microcracks	Absence of microcracks	Total
Before instrumentation	1,681 (11.5)	12,959 (88.5)	14,640 (100)
After instrumentation	1,681 (11.5)	12,959 (88.5)	14,640 (100)
Total	3,362 (11.5)	25,918 (88.5)	29,280 (100)

Data presented as *n* (%).

Discussion

Mandibular molars have shown a mean root canal curvature >20° in morphological studies.[16,17] However, many studies on dentinal microcracks in mandibular molars have evaluated teeth with a mesial root curvature of 10–20°.[9,10,18] Thus, mandibular molars with a mesial root curvature >20° were used in this study.

This study evaluated the effects of the sequential rotary (2S), reciprocating (EFX1) and single-file rotational (NN) systems on microcrack formation in the curved root canals of mandibular molars. Although dentinal microcracks were observed in all 3 groups after root canal instrumentation, these microcracks were present on the micro-CT images obtained before root canal instrumentation as well. Thus, no new microcracks were formed after root canal instrumentation with the 2S, EFX1 and NN systems. This finding is in agreement with the results of the majority of previous studies that used micro-CT for the assessment of microcrack formation after root canal instrumentation.[9,10,12,19–25] A study conducted in 2014 used micro-CT for the first time and showed no cause-and-effect relationship between dentinal microcrack formation and root canal instrumentation.[10] After that, many micro-CT studies confirmed this finding. In their study on human cadavers, De-Deus et al. evaluated 19,060 cross-sectional micro-CT images of maxillary first

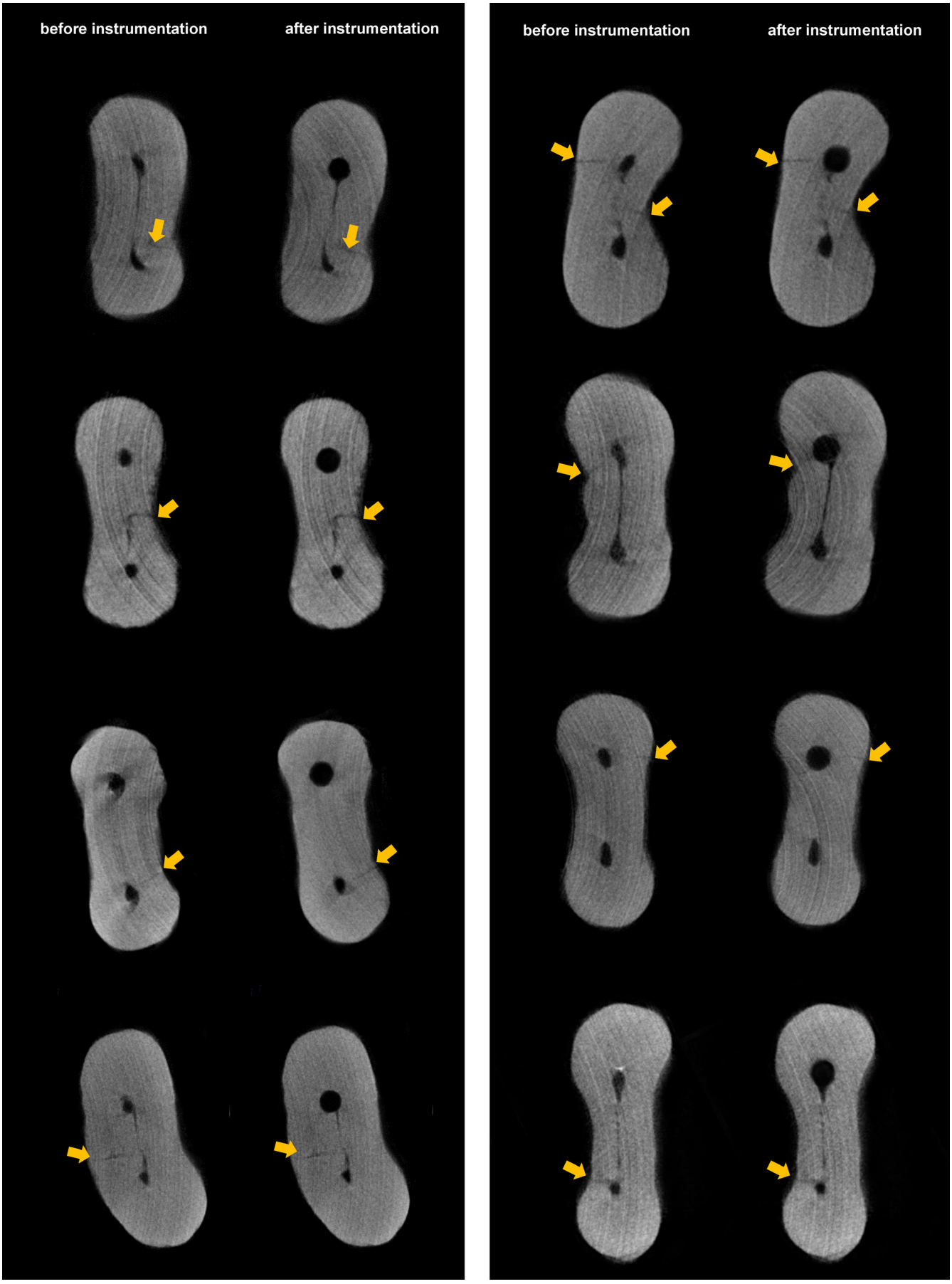

Fig. 3. Representative cross-sectional images of the mesial roots of mandibular molars, showing the presence of cracks (arrows) before and after the preparation of the mesiobuccal canals

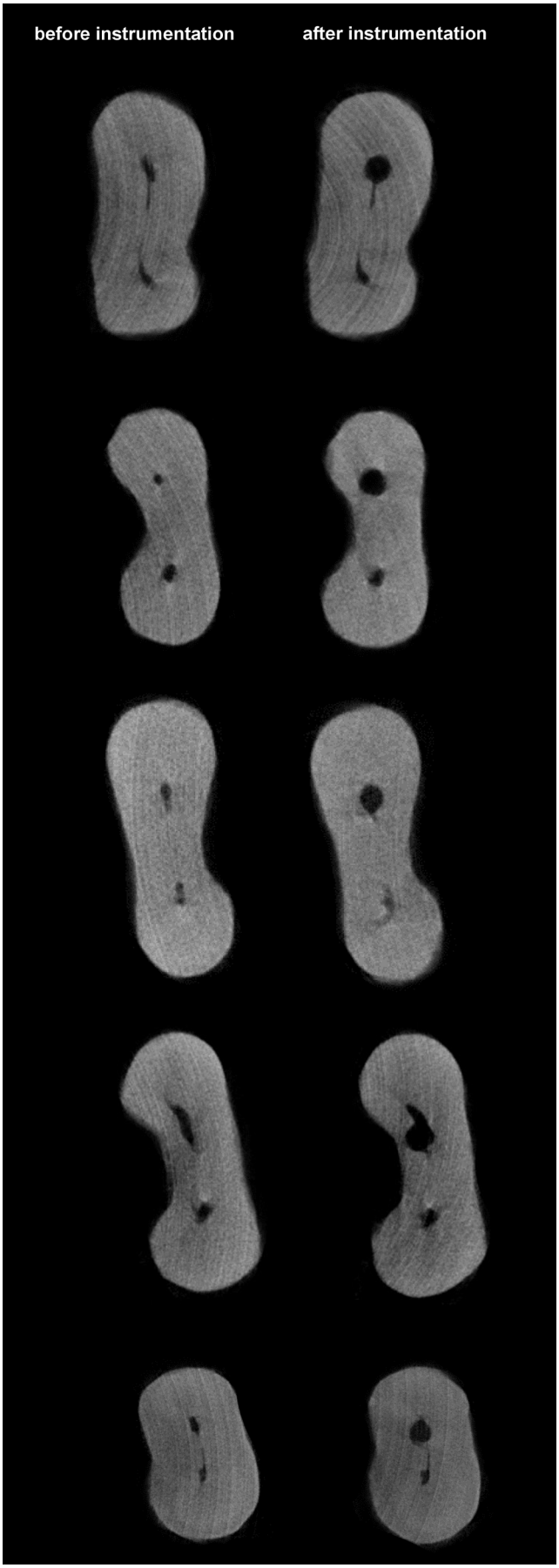

Fig. 4. Representative cross-sectional images of the roots, showing the absence of cracks before and after instrumentation

and second premolars and observed that root canal preparation with rotary and Reciproc files did not cause new dentinal microcracks.[21] In another study, De-Deus et al. evaluated the effects of the ProTaper Next and Twisted File Adaptive systems on dentinal microcrack formation with the use of micro-CT.[9] They evaluated 25,820 cross-sectional images of the mesial roots of mandibular molars before and after instrumentation and found no new microcracks after instrumentation.[9] Another recent study compared root canal preparation with the ProTaper Next, ProTaper Gold and WaveOne Gold systems with regard to the formation of dentinal microcracks in mandibular molars with a root curvature >20°.[12] All the microcracks detected after instrumentation on micro-CT images were present on the pre-instrumentation images as well, and no new microcracks were observed.[12] An in vivo study on intact premolars scheduled for extraction as part of orthodontic treatment evaluated with micro-CT the effects of manual instrumentation and rotary instrumentation with ProTaper files.[22] They found no new dentinal microcracks after root canal instrumentation.[22]

On the contrary, some micro-CT studies have reported the formation of new microcracks after root canal preparation.[2,3,18,26] Ceyhanli et al. showed an increase in the number of dentinal microcracks after the preparation of the mesial roots of mandibular molars with all 3 systems of ProTaper Universal, RaCe and SafeSider, using micro-CT.[2] They reported the maximum number of microcracks in the ProTaper Universal group.[2] Also, Li et al. demonstrated an increase in the length of microcracks present in the mesial roots of mandibular first molars with a curvature of 10–20° after root canal instrumentation.[18] They used single-file systems and showed that the change in microcrack length in the OneShape group was greater than that in the WaveOne and Reciproc systems.[18] Bayram et al. determined that root canal instrumentation with ProTaper Universal significantly increased the formation of new dentinal microcracks.[3] Jamleh et al. reported that both the WaveOne reciprocating and ProTaper Universal rotary systems resulted in the formation of new microcracks.[26]

Controversy in the results may be related to differences in the methodologies and quality of tools used. For instance, in the study by Ceyhanli et al.,[2] the reason for the increase in the number of microcracks might be the fact that only 10 root sections were evaluated (<1.5% of all the obtained images); this may have led to false positive results due to the presence of noise or artifacts.[27] Jamleh et al. required dry and dehydrated surfaces to measure root surface strain as well as for the application of the staining technique,[26] which increased the risk of formation of defects and dentinal microcracks.[28] Another influential factor in microcrack formation is the working length of the root canal. A recently published systematic review indicated that in all root canal preparation techniques, a proper working length (1 mm short of the apex) is required to prevent microcrack formation.[23] This was

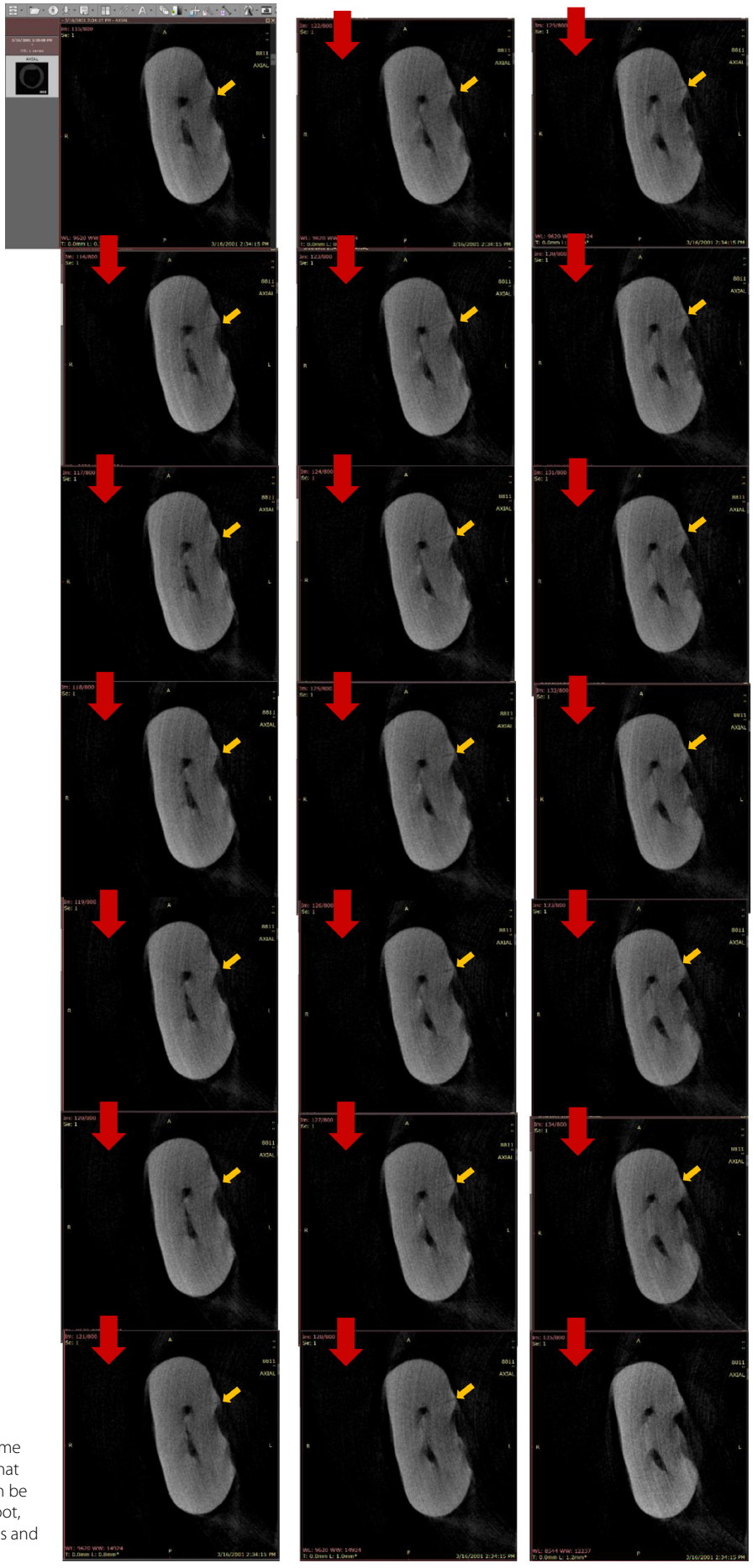

Fig. 5. Sequence of cross-sectional images of the same mesial root of a mandibular molar, demonstrating that the development of microcracks (yellow arrows) can be evaluated throughout the longitudinal axis of the root, confirming the identification of true dentinal defects and artifacts

not the case in the studies by Li et al.[18] and Bayram et al.,[3] and might be the possible reason for the development of dentinal microcracks in their studies.

Studies that used the sectioning technique for the assessment of dentinal microcracks have reported controversial results. The majority of such studies have reported the formation of dentinal microcracks following the use of different instrumentation techniques.[4,23] However, there is no consensus among such studies, and the prevalence of microcrack formation following the use of different instrumentation techniques ranges from 0% to 80%.[4] According to Versiani et al., such a wide range of variation in the prevalence of microcracks may be attributed to the use of different systems and root canal preparation protocols, methods of observation of dentinal defects (with or without a dye), root canal anatomy, the irrigation protocol, and the type of classification used for defects.[4] In sectioning studies, 3–4 sections of the root are often evaluated, made at 1, 3, 7, and 9 mm, or 2, 4, 6, and 8 mm from the apex. This is among the most important drawbacks of the sectioning technique, as the assessment of such a limited number of slices increases the risk of missing a high number of defects along the root canal wall. However, with micro-CT, hundreds of slices of each root can be evaluated. Moreover, micro-CT has a much higher resolution than stereomicroscopy.[5] Another important drawback is the destructive nature of the sectioning method. Stringheta et al. compared the sectioning and micro-CT methods for the detection of dentinal microcracks after root canal instrumentation.[29] They inspected the micro-CT scans before sectioning and noticed that the sectioning method was destructive, and resulted in the formation of new dentinal defects.[29] For this reason, the majority of studies that used the sectioning technique have reported new dentinal crack formation after the instrumentation process.[30] The advantages of micro-CT include its non-destructive nature, its ability to exactly locate dentinal cracks in the root and its three-dimensional (3D) assessment. This allows the evaluation of hundreds of cross-sectional images of the entire root length and the detection of microcracks already present in the root canal walls prior to instrumentation or at different stages of endodontic treatment.[9,24]

The kinematics of the instrument motion and its effect on the formation of dentinal microcracks is another topic of interest that has been addressed in previous studies. A meta-analysis that mainly focused on sectioning studies reported that a reciprocating motion produced a significantly smaller number of dentinal cracks as compared to a rotational motion.[30] However, 2 recently published systematic reviews on micro-CT studies found no correlation between instrumentation kinematics and the formation of dentinal microcracks,[23,24] which confirms the findings of the present study regarding no correlation between the type of instrument motion and dentinal microcrack formation.

Conclusions

According to the results of this in vitro study, the instrumentation of curved root canals with the 2S, EFX1 and NN systems does not result in the formation of new dentinal microcracks.

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Biocompatibility assessment of different root-end filling materials implanted subcutaneously in rats: An in vivo study

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Abstract

Background. Root-end filling materials are used in surgical endodontic treatment to seal the teeth periapically. Ideally, these materials should prevent bacterial leakage by tightly sealing the canal, be biocompatible with the periapical tissues, and preferably stimulate the regeneration of dentin, contributing to the success of treatment.

Objectives. The purpose of this study was to evaluate and compare the biocompatibility of the GuttaFlow® Bioseal cement in relation to MTA Angelus® and Zical® after implantation into the subcutaneous tissue of rats.

Material and methods. Eighteen male albino rats were used in the study. Four polyethylene tubes were implanted in the backs of the rats (3 tubes containing the test materials and 1 empty tube as a control). Nine animals were sacrificed at each interval of 7 and 30 days, and the implants were removed with the surrounding tissue. The samples were evaluated for stromal inflammatory response, fibrous tissue formation, vascular reactivity, and the presence of multinucleated giant cells (MNGCs).

Results. On day 7, the capsules in all subgroups revealed moderate to severe inflammatory reactions with the presence of inflammatory cells, multiple irregular collagen fibers, dilated blood vessels, and MNGCs. However, on day 30, tissue organization was more evident with a reduction in the inflammatory response. In this time interval, the tissue in contact with GuttaFlow Bioseal showed progressive healing with a well-formed fibrous capsule. Conversely, the tissue close to MTA Angelus revealed a fibrous capsule of limited organization with mild pericapsular fibrosis and vascular congestion. Zical showed a mild to moderate persistent inflammatory reaction and vascular reactivity.

Conclusions. The 3 cements demonstrated more severe irritation at the beginning that became milder with time. GuttaFlow Bioseal yielded better tissue organization than MTA Angelus and Zical. Thus, these findings strongly suggest that GuttaFlow Bioseal is a promising material for root-end filling.

Keywords: subcutaneous tissue, bioseal, GuttaFlow, MTA Angelus, Zical

Introduction

The majority of unsuccessful endodontic treatment is the result of the irritants leaking from the infected root canal into the periapical tissues. When non-surgical treatment is unsuccessful or contraindicated, the only solution to save the tooth is via a surgical intervention.[1,2] Surgical endodontic treatment involves root-end preparation followed by the placement of an appropriate root-end filling material. An ideal root-end filling material should provide a tight hermetic seal to prevent microleakage, which could further contaminate the periapical tissues.[3] These materials are in direct contact with the tissues, and therefore, must be biocompatible to avoid further irritation and possible treatment failure.[4] Preferably, the root-end filling material should have the ability to stimulate the periodontium to regenerate while also being bacteriostatic or bactericidal to help accelerate the healing process and reduce the failure rate.[3,5] The material should also be non-toxic, non-carcinogenic and dimensionally stable.[2,3]

The polydimethylsiloxane cements which contain very finely ground gutta-percha are used as cold filling systems for root canals; this formulation is commercially available as GuttaFlow®. Such cements have been introduced to overcome the disadvantages of warm obturation techniques.[6] GuttaFlow is bioactive due to the addition of silica, calcium oxide and phosphorous oxide particles; it has been introduced onto the market as GuttaFlow Bioseal. The substances added to the cement play a role in the stimulation of tissue regeneration and healing.[7] GuttaFlow Bioseal is characterized by low solubility, high bond strength, minimal calcium release, and alkalinizing activity.[8–10]

Mineral trioxide aggregate (MTA) is the best known bioactive cement in the endodontic field. This cement is widely used in different endodontic treatment, including root-end surgeries, root perforation repair, internal resorption, pulp capping, apexification, and obturation. It is mainly composed of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, dehydrated calcium sulfate, and bismuth oxide. Its bioactivity is due to the rise in pH to 12.5 that occurs after 3 h of mixing, which subsequently stimulates interleukin production and calcium ion release.[11] Mineral trioxide aggregate has been found to stimulate hard tissue deposition when applied as a retro-filling material in endodontically treated dogs' teeth.[12] There are several drawbacks to the use of MTA, including a long setting time, difficult handling, low strength, and a high cost.[13]

Traditionally, zinc oxide–eugenol (ZnO/E) cements have been among the most commonly used and recommended cements with a long history of successful use. Despite their positive characteristics, they have several shortcomings that have favored their replacement, including a long setting time, excessive solubility and the lack of adhesion.[10,14,15]

Accordingly, this study aimed to evaluate and compare the biocompatibility promoted by the polydimethylsiloxane-gutta-percha calcium silicate-containing cement (GuttaFlow Bioseal) in relation to MTA (MTA Angelus®) and ZnO/E (Zical®) after implantation into the subcutaneous connective tissue of rats.

Material and methods

The cements used in the study were GuttaFlow Bioseal (Coltène/Whaledent, Altstätten, Switzerland), MTA Angelus (Angelus, Londrina, Brazil) and Zical (Prevest DenPro Limited, Jammu, India). The composition of the materials is described in Table 1.

Animal study design

This experiment was conducted in the Animal House of the Faculty of Medicine, Cairo University, Egypt, according to the guidance and approval of the Institutional Animal Care & Use Committee of Cairo University (CU-IACUC) (approval No. CU III F 7718).

Eighteen adult male albino rats with an average weight of 150–200 g were provided by the Animal House of the Faculty of Medicine, Cairo University, Egypt. The animals

Table 1. Endodontic cements used in the study

Commercial name	Manufacturer	Presentation	Composition	Lot No.
GuttaFlow Bioseal	Coltène/Whaledent, Altstätten, Switzerland	dual-barrel syringe	gutta-percha powder particles, polydimethylsiloxane, platinum catalyst, zirconium dioxide, calcium salicylate, nanosilver particles, paraffin, coloring, and bioactive glass ceramic	H84160
MTA Angelus	Angelus, Londrina, Brazil	powder and liquid	powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, dehydrated calcium sulfate, and bismuth oxide; liquid:distilled water	101648
Zical	Prevest DenPro Limited, Jammu, India	powder and liquid	powder: zinc oxide, bismuth subcarbonate, barium sulfate, sodium borate, iodoform, and hydrogenated resin; liquid: eugenol	1521802

were kept in an aerated chamber with 12-h dark/light intervals, divided according to the study period. The cages were cleaned on a daily basis, and the rats were allowed unlimited access to food and water. The animals were randomly divided into 2 groups (n = 9 in each group), according to the sacrifice dates (7 days and 30 days). These groups were further subdivided into 4 subgroups according to the material used:

– subgroup 1 – polyethylene tubes filled with GuttaFlow Bioseal (n = 9);
– subgroup 2 – polyethylene tubes filled with MTA Angelus (n = 9);
– subgroup 3 – polyethylene tubes filled with Zical (n = 9);
– subgroup 4 – empty polyethylene tubes (control) (n = 9).

The materials were mixed under aseptic conditions, according to the manufacturers' instructions. Sterile polyethylene tubes were filled with GuttaFlow Bioseal or MTA Angelus and set within 15 min after being prepared, Zical, or left empty. They were immediately implanted into the dorsal subcutaneous tissue of the animals.

The back of each animal was shaved in 4 areas (upper right, upper left, lower right, and lower left) and disinfected with 10% betadine antiseptic solution (Mundipharma Egypt co., Cairo, Egypt). The animals were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg of body weight (b.w.)) combined with xylazine hydrochloride (8 mg/kg b.w.). Incisions of 2 cm in length were made in the head-tail orientation, creating 2 pockets on each side of the back of each rat (Fig. 1). After the polyethylene tubes were implanted, the skin was closed using 4/0 silk sutures (International Sutures Manufacturing Co., Cairo, Egypt). Postoperatively, each animal received 10 mg/kg b.w. Flumox® (EIPICO, Tenth of Ramadan City, Egypt) intramuscularly to avoid secondary bacterial infection, 10 mg/kg b.w. Cataflam® (Novartis, Cairo, Egypt) to avoid any possibility of postoperative pain as well as topical antibiotic spray Bivatracin (ECAP, Cairo, Egypt) to avoid local infection. On days 7 and 30 after implantation, the animals were euthanized by intracardiac overdose of sodium thiopental (80 mg/kg b.w.).[16]

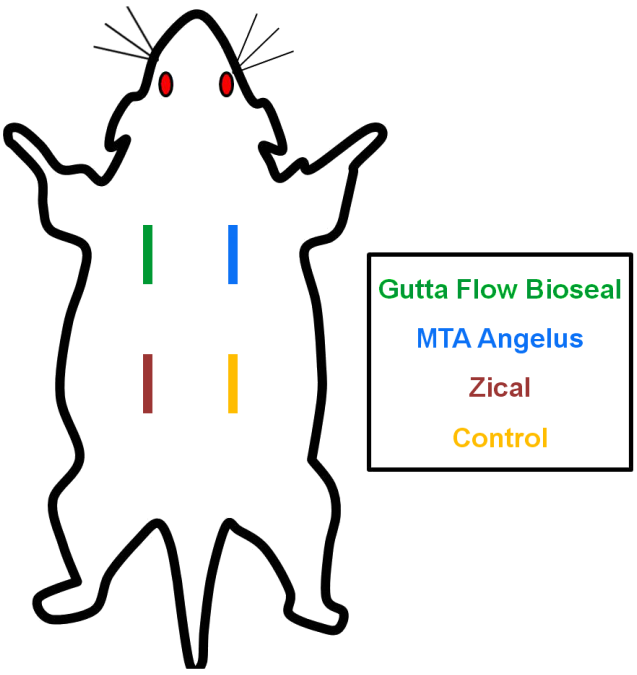

Fig. 1. Diagrammatic presentation of the 4 incision sites and materials implanted into the dorsum of the rats

Histopathologic evaluation

Samples of skin and subcutaneous tissues containing the implants were excised with a safety margin of 1 cm. They were placed in 10% formalin at pH 7.2, buffered with 0.1 M sodium phosphate at room temperature for 24 h to allow fixation. Subsequently, the polyethylene tubes were carefully removed from the samples. Next, the tissues were dehydrated in graded ethanol, treated with xylene and embedded in paraffin. Longitudinal sections, 4-micrometer thick, were stained with hematoxylin and eosin (H&E) for morphological and morphometrical analyses.

The tissue reaction at the end of the tubes was scored according to a previous study, including stromal inflammatory response (mononuclear cells), fibrous tissue formation, vascular reactivity, and the presence of multinucleated giant cells (MNGCs) (Table 2).[17] The histopathological evaluation was performed using light mi-

Table 2. Criteria for scoring the severity of reaction

Criterion	0	1	2	3
Stromal inflammatory response	– no reaction – few inflammatory cells	– mild reaction – less than 25 inflammatory cells	– moderate reaction – 25–125 inflammatory cells	– severe reaction – more than 125 inflammatory cells
Fibrous tissue formation	– no reaction – normal collagen fiber morphology	– mild reaction – mild collagen fiber irregularity	– moderate reaction – moderate collagen fiber irregularity	– severe reaction – severe collagen fiber irregularity
Vascular reactivity	– no reaction – no significant vascular proliferation	– mild reaction – number of vascular structures in 1 high-power field (×40) <25	– moderate reaction – number of vascular structures in 1 high-power field (×40) 25–50	– severe reaction – number of vascular structures in 1 high-power field (×40) >50
MNGCs	absent	present	present	present

MNGCs – multinucleated giant cells.

croscopy (Leica Microsystems, Heerbrugg, Switzerland) under ×400 magnification, while the analyses of the connective tissue capsule thickness,[18] the number of cell layers in the capsule[19] and the collagen fiber diameter were performed on images acquired with ×100 magnification. Five fields were measured from each sample and the mean values were calculated.

The Fiji ImageJ software (https://imagej.net/software/fiji/)[20] was utilized. The data was obtained using the Leica Qwin 500 Image Analysis Software (Leica Microsystems). The image analyzer consisted of a colored video camera, a colored monitor, and the hard drive of an IBM personal computer (IBM Corp., Armonk, USA) connected to the microscope controlled by the Leica Qwin 500 software.

Statistical analysis

The statistical analysis of the tissue response was performed at day 7 and day 30. Differences in the grades assigned to particular subgroups and between the 2 experimental periods were presented as frequency, and were evaluated using the Mann–Whitney *U* test and Kruskall–Wallis non-parametric test followed by a post hoc test. The thickness of the connective tissue capsule, the number of cell layers in the capsule and the collagen fiber diameter were analyzed using the two-way analysis of variance (ANOVA) followed by a post hoc test. The level of significance was set at 0.05.

Results

In all 4 subgroups in both time periods, mild to severe inflammation, vascular reactivity, fibrous tissue formation, and the presence of MNGCs were observed (Table 3, 4; Fig. 2, 3).

Microscopic analysis of the tissue–material interface

On day 7, all subgroups showed moderate to severe stromal inflammatory response on the capsule at the tissue–material interface. The experimental subgroups displayed inflammatory cell infiltration, mainly lymphocytes and macrophages, with increased levels of new blood vessel formation around them. The connective tissue was poorly organized with a few MNGCs (Fig. 2A–D). The remnants of the implanted material were observed in the MTA Angelus subgroup (Fig. 2B). As compared to the experimental subgroups, the control subgroup showed more organized connective tissue with moderate macrophage and lymphocyte infiltration as well as a small fibrin clot (Fig. 2D).

Table 3. Comparison of the *p*-values of the subgroups at the 2 time periods for the criteria assessed

Criterion	Day	GuttaFlow Bioseal	Day	MTA Angelus	Day	Zical	Day	Control	Day	*p*-value (between subgroups)
Stromal inflammatory response	7^{b}	0.028*	7^{b}	0.011*	7^{a}	0.003*	7^{b}	0.011*	7	0.006*
	30^{b}		30^{b}		30^{a}		30^{b}		30	0.004*
Fibrous tissue formation	7	0.073	7	0.150	7	0.033*	7	0.298	7	0.065
	30		30		30		30		30	0.442
Vascular reactivity	7^{a}	0.001*	7^{a}	0.011*	7^{a}	0.008*	7^{b}	0.140	7	0.003*
	30^{b}		$30^{a,b}$		30^{a}		30^{b}		30	0.018*
MNGCs	7^{a}	0.028*	7^{b}	1.000	7^{a}	0.028*	7^{b}	1.000	7	0.019*
	30		30		30		30		30	1.000

* – statistically significant. Within the same row (whenever the difference between the subgroups is statistically significant), the same superscript letter means no statistically significant difference.

Table 4. Scoring of histopathologic events observed in each subgroup at the 2 time periods

Criterion	Day	*n*	GuttaFlow Bioseal				MTA Angelus				Zical				Control			
			G0	G1	G2	G3	G0	G1	G2	G3	G0	G1	G2	G3	G0	G1	G2	G3
Stromal inflammatory response	7	9	0	5	4	0	0	4	5	0	0	0	5	4	0	4	5	0
	30	9	0	9	0	0	0	9	0	0	0	5	4	0	0	9	0	0
Fibrous tissue formation	7	9	0	3	6	0	0	3	5	1	0	1	6	2	0	6	3	0
	30	9	0	7	2	0	0	6	3	0	0	5	4	0	0	8	1	0
Vascular reactivity	7	9	0	0	6	3	0	0	6	3	0	0	4	5	0	5	4	0
	30	9	0	7	2	0	0	4	5	0	0	2	7	0	0	8	1	0
MNGCs	7	9	5	4	0	0	9	0	0	0	5	4	0	0	9	0	0	0
	30	9	9	0	0	0	9	0	0	0	9	0	0	0	9	0	0	0

G – grade.

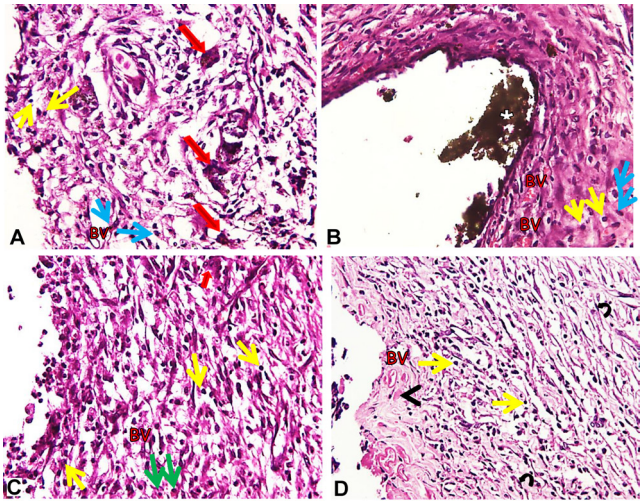

Fig. 2. Photomicrographs of hematoxylin and eosin (H&E) staining sections showing a portion of the capsule at the tissue–material interface on day 7

A – GuttaFlow Bioseal; B – MTA Angelus; C – Zical; D – control; ×400 magnification.
The capsules show a moderate to severe inflammatory reaction. The capsules exhibit several inflammatory cells, mainly lymphocytes (yellow arrows), plasma cells (blue arrows) and macrophages (green arrows). Multinucleated giant cells (MNGCs) (red arrows) and blood vessels (BV) are observed. Fibroblastic activity (curved arrow) and minimal fibrinoid-like deposition (black-arrow head) are observed in 2D.

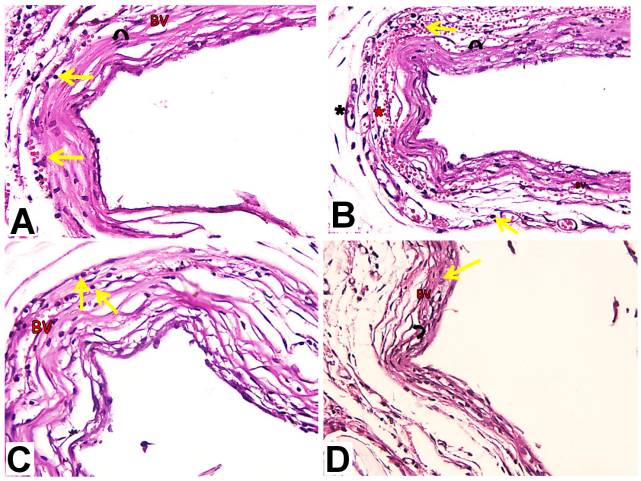

Fig. 3. Photomicrographs of hematoxylin and eosin (H&E) staining sections showing a portion of the capsule at the tissue–material interface on day 30

A – GuttaFlow Bioseal; B – MTA Angelus; C – Zical; D – control; ×400 magnification.
The fibrous capsule shows mild inflammatory cell infiltration in 3A, 3B and 3D. Mild pericapsular fibrosis (red asterisks) and congested capillaries (black asterisks) are observed in 3B. Mild to moderate inflammation is observed in 3C. The remodeling of the fibrous capsule and collagen fibers with marked fibroblastic activity (curved arrow) is observed in 3D

On day 30, all subgroups showed a mild to moderate inflammatory tissue response on the capsule at the tissue–material interface. The GuttaFlow Bioseal subgroup showed progressive healing with a well-formed fibrous capsule; it also exhibited multiple parallel collagen fibers with a marked decrease in inflammatory cell infiltration (Fig. 3A). The MTA Angelus subgroup exhibited a fibrous capsule of limited organization with mild peri-capsular fibrosis, including some congested capillaries (Fig. 3B). The Zical subgroup showed significantly more inflammatory cells, vascular reactivity and collagen fiber irregularity as compared to other subgroups (Fig. 3C). The control subgroup displayed more organized tissue with predominant connective tissue fibers, fibroblasts and inflammatory reactions (Fig. 3D). Fibroblasts were observed among the inflammatory cells and blood vessels in all subgroups (Fig. 3A–D). Multinucleated giant cells were absent in all subgroups.

Considering the severity of the inflammatory reaction displayed by the subgroups, there were statistically significant differences in stromal inflammatory response between the subgroups on day 7 and day 30 ($p = 0.006$ and $p = 0.004$, respectively), with a larger number of inflammatory cells in the Zical subgroup on both observation days. There were no statistically significant differences in fibrous tissue formation between the subgroups on day 7 and day 30 ($p = 0.065$ and $p = 0.442$, respectively). There were statistically significant differences in vascular changes between the subgroups on day 7 and day 30 ($p = 0.003$ and $p = 0.018$, respectively), with the greatest amount of vascular changes recorded in the Zical subgroup. There were statistically significant differences in MNGCs between the subgroups on day 7 ($p = 0.019$), but not on day 30 ($p = 1.000$) (Table 3).

Comparing the 2 observation times, stromal inflammatory response decreased statistically significantly between the time periods for the GuttaFlow Bioseal, MTA Angelus, Zical, and control subgroups ($p = 0.028$, $p = 0.011$, $p = 0.003$, and $p = 0.011$, respectively). Although it was not statistically significant, fibrous tissue formation decreased over time for the GuttaFlow Bioseal, MTA Angelus and control subgroups ($p = 0.073$, $p = 0.150$, $p = 0.298$, respectively). Conversely, a statistically significant decrease was recorded on day 30 in the Zical subgroup ($p = 0.033$). There was a statistically significant decrease in vascular reactivity between the time periods for the GuttaFlow Bioseal, MTA Angelus and Zical subgroups ($p = 0.001$, $p = 0.011$ and $p = 0.008$, respectively), while the control subgroup showed no significant difference between the 2 observation times ($p = 0.140$). There was a statistically significant difference between the 2 observation times for MNGCs in the GuttaFlow Bioseal and Zical subgroups ($p = 0.028$ and $p = 0.028$, respectively), but this difference was not statistically significant for the MTA Angelus and control subgroups ($p = 1.000$ and $p = 1.000$, respectively) (Table 3).

The connective tissue capsule thickness and the number of cell layers in the capsule decreased, while the collagen fiber diameter increased in all subgroups with regard to both time periods. An increase in the collagen fiber diameter was not statistically significant ($p = 0.101$), but decreases in the connective tissue capsule thickness and the number of cell layers in the capsule were statistically significant ($p < 0.05$) (Table 5).

On day 7, the capsules exhibited a well-defined structure of variable thickness; the highest mean capsule thickness was detected in the Zical subgroup, while the lowest value was detected in the control subgroup on day 30 (Table 5).

Table 5. Comparison of the subgroups at the 2 time periods in terms of particular parameters

Parameter	Day	Control	Zical	MTA Angelus	GuttaFlow Bioseal	*p*-value
Connective tissue capsule thickness [μm]	7	103.15 ±10.72C,D	323.53 ±70.38A	236.28 ±22.60B	119.64 ±20.20C	0.000*
	30	48.18 ±12.74E	72.30 ±15.71D,E	69.89 ±6.58D,E	59.79 ±10.71E	
Number of cell layers (n)	7	4.67 ±1.32C,D	9.17 ±1.00A	9.06 ±0.53A	8.33 ±0.71A	0.000*
	30	4.39 ±0.70D	6.11 ±1.05B	5.78 ±0.71B,C	4.72 ±0.83C,D	
Collagen fiber diameter [μm]	7	1.96 ±0.92	4.61 ±0.69	4.66 ±1.03	2.62 ±1.32	0.101
	30	2.45 ±1.25	6.64 ±0.82	5.12 ±1.59	4.30 ±1.40	

Data presented as mean ± standard deviation (*M ±SD*). * – statistically significant. The same superscript letter means no statistically significant difference.

The pairwise comparison revealed statistically significant differences between the Zical subgroup on day 7 and the following subgroups: control on day 7 ($p < 0.05$); control on day 30 ($p < 0.05$); GuttaFlow Bioseal on day 7 ($p < 0.05$); GuttaFlow Bioseal on day 30 ($p < 0.05$); MTA Angelus on day 7 ($p < 0.05$); and MTA Angelus on day 30 ($p < 0.05$).

Furthermore, the pairwise comparison showed statistically significant differences between the Zical subgroup on day 30 and the following subgroups: Zical on day 7 ($p < 0.05$); GuttaFlow Bioseal on day 7 ($p = 0.018$); and MTA Angelus on day 7 ($p < 0.05$).

A significant relationship was also detected between the MTA Angelus subgroup on day 7 and the control subgroup on day 7 ($p < 0.05$), the control subgroup on day 30 ($p < 0.05$), the GuttaFlow Bioseal subgroup on day 7 ($p < 0.05$), and the GuttaFlow Bioseal subgroup on day 30 ($p < 0.05$). Moreover, there was a significant relationship between the MTA Angelus subgroup on day 30 and the MTA Angelus subgroup on day 7 ($p < 0.05$) and the GuttaFlow Bioseal subgroup on day 7 ($p = 0.010$).

Additionally, a significant relationship was detected between the GuttaFlow Bioseal subgroup on day 7 and the control subgroup on day 30 ($p < 0.05$). There was also a significant relationship between the GuttaFlow Bioseal subgroup on day 30 and the GuttaFlow Bioseal subgroup on day 7 ($p = 0.001$) and the control subgroup on day 7 ($p = 0.040$).

Moreover, there was a significant relationship between the control subgroup on day 30 and the control subgroup on day 7 ($p = 0.003$).

Regarding the number of cell layers in the capsule, the highest mean number of cell layers was detected in the Zical subgroup on day 7, while the lowest number of cell layers was detected in the control subgroup on day 30; this difference was statistically significant ($p < 0.05$).

The pairwise comparison revealed a statistically significant relationship between the Zical subgroup on day 7 and the control subgroup on day 7 ($p < 0.05$), the control subgroup on day 30 ($p < 0.05$), the MTA Angelus subgroup on day 30 ($p < 0.05$), and the GuttaFlow Bioseal subgroup on day 30 ($p < 0.05$). There was also a statistically significant relationship between the Zical subgroup on day 30 and the Zical subgroup on day 7 ($p < 0.05$), the control subgroup on day 7 ($p = 0.021$), the control subgroup on day 30 ($p = 0.030$), the GuttaFlow Bioseal subgroup on day 7 ($p < 0.05$), the GuttaFlow Bioseal subgroup on day 30 ($p = 0.031$), and the MTA Angelus subgroup on day 7 ($p < 0.05$).

A statistically significant relationships was also detected between the MTA Angelus subgroup on day 7 and the control subgroup on day 7 ($p < 0.05$), the control subgroup on day 30 ($p < 0.05$) and the GuttaFlow Bioseal subgroup on day 30 ($p < 0.05$). There was also a statistically significant relationship between the MTA Angelus subgroup on day 30 and the MTA Angelus subgroup on day 7 ($p < 0.05$), the control subgroup on day 30 ($p = 0.031$) and the GuttaFlow Bioseal subgroup on day 7 ($p < 0.05$).

Additionally, a significant relationship was detected between the GuttaFlow Bioseal subgroup on day 7 and the control subgroup on day 7 ($p < 0.05$), the control subgroup on day 30 ($p < 0.05$) and the GuttaFlow Bioseal subgroup on day 30 ($p < 0.05$).

There was a non-significant increase in the collagen fiber diameter among the subgroups with time.

Discussion

The biocompatibility of root-end filling cements is one of the most important requirements of the material, since it comes into direct contact with the vital periradicular tissues. In vivo subcutaneous implantation is considered one of the most reliable procedures to assess the biocompatibility of dental materials.[21] The endodontic cement extruded from the polyethylene tube orifice creates a tissue–tube interface, which triggers inflammatory response similar to that found at the periapical area of an endodontically treated tooth.[22] Inert polyethylene tubes were used for implantation due to their ability to hold the tested material in direct contact with the tissues.[21] An additional empty tube was implanted as a control in an attempt to control variables, avoid selection bias, and neutralize any confounders that might affect the results.[16]

In this study, stromal inflammatory response (mononuclear cells), vascular changes, fibrous tissue formation, and the presence of MNGCs in the subcutaneous tissues of the rats were evaluated. In all subgroups, the results showed a moderate to severe stromal inflammatory response presented as the recruitment of inflammatory cells, numerous blood vessels and irregular collagen fibers with the presence of MNGCs. Following the initial moderate to severe

reactions, all tissue reactions decreased over time. In addition, the structural reorganization of the capsules was demonstrated over time. This is a positive indicator of the material tolerability, as it is considered an immune reaction that produces foreign bodies recognized as harmless.[23]

Besides the recruitment of inflammatory cells, angiogenesis is essential for fibroblast proliferation and initial granulation tissue formation.[24] The number of blood vessels decreased with time, which may be directly associated with the regression of the inflammatory reaction and the rearrangement of the connective tissue. On day 30, the control subgroup showed the least number of blood vessels, followed by the GuttaFlow Bioseal and MTA Angelus subgroups; the greatest values were associated with Zical.

Multinucleated giant cells could be observed, as demonstrated in Fig. 2A and 2C. These giant cells disappeared with time over the course of the study. These findings are consistent with the results of another in vivo study by Ghanaati et al.[25] In their study, the number of MNGCs decreased with time as the degradation of the material progressed, suggesting that MNGCs were the main phagocytic cells associated with the degradation of the material.[25] Additionally, Hernandez-Pando et al. proposed that MNGCs contributed to the initiation and maintenance of the inflammatory process, and might also be involved in the downregulation of inflammation and the induction of the fibrotic process via the production of proinflammatory and anti-inflammatory cytokines, depending on the inflammatory process phase.[26] Notably, it is possible that the apoptotic cell death mechanism was involved in the elimination of MNGCs in the tissues.[26]

GuttaFlow Bioseal promoted connective tissue remodeling similar to that observed in the control subgroup, which indicates that the material was well tolerated by the tissues. It induced the formation of the collagenous capsule containing blood vessels and fibroblasts with mild inflammation 30 days after subcutaneous implantation. In previous studies, GuttaFlow cements (GuttaFlow and GuttaFlow 2) showed low cytotoxicity,[27,28] which is an essential requirement for endodontic cements that come in contact with vital tissues. GuttaFlow Bioseal has been found to be slightly soluble when in contact with water due to the presence of soluble bioactive particles. This solubility is within the American Dental Association (ADA) specifications and has been suggested to provide the necessary ions needed for the remineralization of dentin.[10] In an in vitro study on GuttaFlow Bioseal cultured on human periodontal ligament stem cells (hPLSCs), GuttaFlow showed the least cytotoxicity, maintained cell viability, and exhibited better cell migration, morphological characteristics and cytoskeletal organization patterns. In another in vitro study, GuttaFlow Bioseal was found to be the most biocompatible when cultured on mouse fibroblast cells as compared to GuttaFlow 2, AH Plus and MTA Fillapex.[7]

MTA Angelus showed the accentuated recruitment of inflammatory cells on day 7. This elevated inflammatory reaction in the initial assessment period may be due to the high alkaline pH reached by the cement during setting, leading to the production and release of proinflammatory cytokines.[29] Additionally, MTA has shown a cytotoxic effect on V79 fibroblasts and BALB/c 3T3 cells, which may be due to the presence of toxic components, such as salicylate and diluting resins.[30] This is in accordance with the findings of this study, showing that the MTA Angelus subgroup demonstrated inflammatory cell infiltration on day 7, which decreased until day 30, although it was still evident.[31]

Zical demonstrated the highest inflammatory infiltration throughout the study, which may be attributed to its constituents – zinc oxide ions and eugenol oil. These findings are consistent with an in vivo study showing an intense histopathologic reaction to a ZnO/E sealer throughout the experimental duration, with profuse lymphoplasmacytic infiltrate and large quantities of macrophages.[32] Similarly, ZnO/E endodontic sealers caused a mild to moderate inflammatory reaction with a predominance of lymphocytes subcutaneously in rats, which declined into a mild reaction at the later period of the experiment.[33] The setting reaction of this cement involves the hydrolysis of zinc oxide ions to zinc hydroxide, which chelates with eugenol oil in order to give a relatively soluble matrix of zinc oxide and eugenol with trapped unreacted eugenol.[10] A study on the cytotoxicity of eugenol reported that it had a significant potential for periapical toxicity. Eugenol leaches out into the surrounding periapical tissues, contributing to the development of periapical inflammation, or even the persistence of a pre-existing periapical lesion.[34] Eugenol may inhibit macrophage function by significantly affecting their adherence and potential for phagocytosis, which results in inflammatory reactions in the periapical tissues.[35,36] On day 30, there was a significant reduction in the inflammatory response produced by Zical. This might be explained by the neutralization of the previously liberated eugenol, since Zical is a ZnO/E-based sealer.[35,36]

The control subgroup in both time periods showed inflammatory reactions, and since polyethylene is an inert material, they may have been triggered by trauma from the surgical procedure.[37] Accordingly, the inflammatory reaction brought about by the experimental cements was due to both the surgical procedure and their components released into the surrounding tissues. This observation makes it evident that although the cements did show inflammatory response, they were still considered biocompatible if the level of inflammatory reponse was acceptable and restricted to short periods of time.[38]

Conclusions

Within the limitations of this study, Zical showed the greatest inflammatory reaction, while GuttaFlow Bioseal exhibited the least inflammatory reaction. Hence, it can be concluded that GuttaFlow Bioseal is biocompatible and comparable to MTA Angelus, making it a promising root canal sealer. It is

recommended that other stains are used in future studies for enhanced detection of collagen fibers, such as Masson–Goldner or picrosirius red staining.

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Effect of the temperature of sodium hypochlorite on the cyclic fatigue resistance of ProTaper Gold rotary files

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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. Instrument fracture is one of major challenges during root canal treatment. In order to reduce such errors, it seems necessary to investigate the effects of potentially impactful factors. One of such factors could be the temperature of an agitator.

Objectives. This study examined the effects of different temperature of the sodium hypochlorite solution on the cyclic fatigue resistance of ProTaper Gold (PTG) rotary files.

Material and methods. Forty-five PTG S1 rotary files were tested in a metal block that simulated a canal curvature angle of 60° and a curvature radius of 5 mm. They were randomly divided into 3 groups of 15 according to sodium hypochlorite temperatures of 22°C (group 1), 4°C (group 2) and 37°C (group 3). Files from each group were rotated at 300 rpm in the block at each temperature. The number of cycles to fracture was calculated and the fragment length was measured. The fractured surfaces were examined by means of scanning electron microscopy (SEM). The statistical analysis was completed using the Kolmogorov–Smirnov and Kruskal-Wallis tests, and the IBM SPSS Statistics for Windows software, v. 22.0, at a significance level of 5%.

Results. The cyclic fatigue resistance of the PTG rotary files was not significantly affected by the temperature of sodium hypochlorite ($p > 0.05$).

Conclusions. Increasing the temperature of sodium hypochlorite to 37°C or decreasing it to 4°C did not significantly affect the cyclic fatigue resistance of PTG rotary files.

Keywords: temperature, sodium hypochlorite, rotary instruments, cyclic fatigue, nickel-titanium

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Introduction

One of the most important goals in root canal therapy is to reduce the microbial load in the root canal system to an acceptable level. One important step in achieving this goal is the cleaning and shaping of the root canal.[1] Currently, rotary files are widely used for shaping root canals.[1,2] Research has shown that nickel-titanium (Ni-Ti) rotary files result in fewer procedural errors during the preparation and shaping of root canals, and more favorable outcomes as compared to stainless-steel files.[1–3] However, the use of rotary files may be associated with problems, such as file fracture within the root canal.[4]

The fracture of rotary files within the root canal can have 2 forms – torsional fracture and cyclic fatigue fracture. Torsional fracture occurs when the file tip is engaged in the root canal and the file shank is in rotation, leading to the fracture of the file tip. Cyclic fatigue fracture occurs when the file undergoes repeated stress and tension, and fractures within the root canal. Cyclic fatigue is responsible for the majority of fractures. Many studies have been undertaken in an attempt to reduce the risk of this type of fracture.[4–7]

Root canal therapy is most commonly performed using irrigation solutions, with sodium hypochlorite being the most freqeuntly used.[1] This irrigation solution exhibits high antibacterial activity and strong tissue solubility. Furthermore, according to some studies, its antibacterial activity and tissue solubility increase with an increase in temperature.[5,8] Some studies have reported that the sodium hypochlorite solution can cause the corrosion of Ni-Ti files due to its hypochlorite ion content. This would affect its mechanical properties and increase the odds of a sudden fracture.[9] Other studies, however, have reported that the sodium hypochlorite solution does not have any effect on the structure of heat-treated files.[10]

ProTaper Gold (PTG; Dentsply Tulsa Dental Specialties, Tulsa, USA) is a new generation of ProTaper rotary files. According to the manufacturer, PTG files have been designed based on advanced metallurgy and exclusive tapering, with high efficacy and a safe tip. Due to the heat-treatment process, the shape memory and higher plasticity associated with these files reduce the number of the preparation errors in curved canals. In addition, this generation of files is more flexible than the ProTaper Universal file type.[11,12]

Continuous advances in rotary file systems and their ever-increasing use have made it possible to decrease the number of procedural errors. Also, according to previous studies, an increase in the temperature of the sodium hypochlorite solution reduces the microbial load. On the other hand, a decrease in the file temperature reduces friction and failure during root canal treatment steps.[13,14] A limited number of studies have investigated the effect of temperature variations on the fracture resistance of these files. Therefore, the aim of this study was to investigate the effect of the temperature of the sodium hypochlorite solution on the fracture resistance of PTG rotary files.

Material and methods

Forty-five PTG S1 rotary files were tested in this study. The samples were examined using a scanning electron microscope (SEM) (Leica M205 C; Leica Microsystems, Wetzlar, Germany) for structural defects or deformities. Defective files were replaced by new ones with no structural defects.

The specimens were randomly divided into 3 groups (n = 15). A sodium hypochlorite (Wizard™; Rehber Kimya, Istanbul, Turkey) solution was used at 22°C (room temperature) in group 1, at 4°C in group 2, and at 37°C (body temperature within the root canal) in group 3. The concentration of the sodium hypochlorite solution was 5.25% for all groups.

For the cyclic fatigue test, a stainless-steel metal block with a simulated canal with a curvature angle of 60°, a curvature radius of 5 mm and a length of 25 mm was used (Fig. 1). It was designed in such a way so that the file could move freely within the canal. The file was inserted into a handpiece connected to an endodontic motor (Silver; VDW, Munich, Germany). The block and handpiece were fixed in place with a clamp. The engine speed was set at 300 rpm and a torque of 5.1 N·cm, based on the manufacturer's recommendations. Before starting the procedure, oil was poured into the canal to reduce friction. The file was inserted into the canal up to a length of 25 mm. The block was fixed inside a recipient that was filled with 5.25% sodium hypochlorite. The temperature was preset at 22°C, 4°C and 37°C with a tolerance limit of 1°C. Time was measured with a timer, starting from the moment when the file began to rotate. The timer was stopped a fracture was observed or after hearing a fracture sound, and the time was recorded. The time in minutes was multiplied by 300 rpm to calculate the number of cycles to fracture (NCF). The fragment length was measured under an SEM (Leica M205 C) at ×10 magnification. The 3 fractured instruments were cleaned with absolute alcohol in an ultrasonic bath. The fractured surface was examined using an SEM (Leica M205 C), as demonstrated in Fig. 2–7.

Statistical analysis

The mean (M) and standard deviation (SD) values were calculated in terms of NCF. The distribution of data was abnormal, as confirmed by the Kolmogorov–Smirnov test. The data was analyzed with the Kruskal–Wallis test. A confidence level of 95% was established. The IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA), was used for data analysis.

Fig. 1. Artificial stainless-steel canal

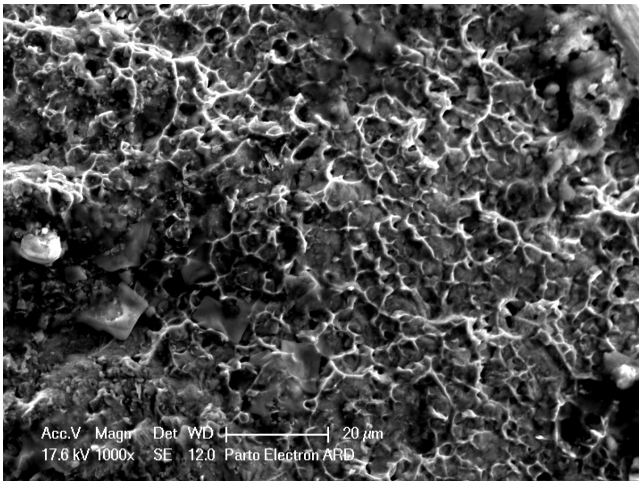

Fig. 3. Scanning electron microscopy (SEM) image of the ProTaper Gold (PTG) S1 instrument after cyclic fatigue testing using NaOCl at 4°C
×1,000 magnification.

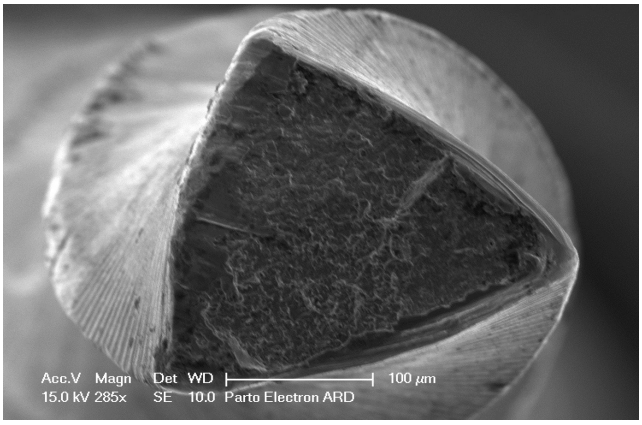

Fig. 4. Scanning electron microscopy (SEM) image of the ProTaper Gold (PTG) S1 instrument after cyclic fatigue testing using NaOCl at 22°C
×285 magnification.

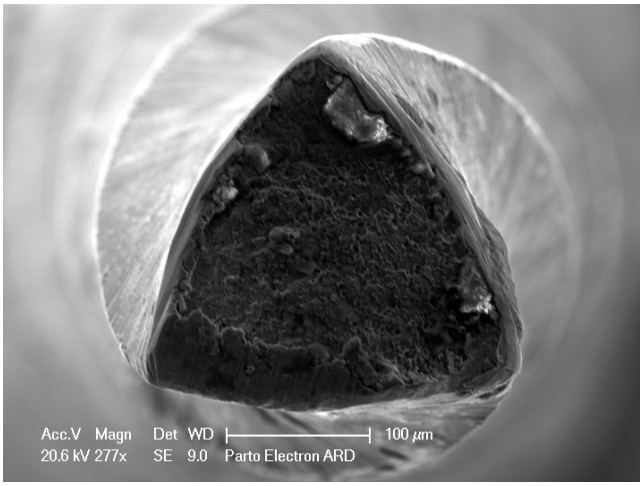

Fig. 2. Scanning electron microscopy (SEM) image of the ProTaper Gold (PTG) S1 instrument after cyclic fatigue testing using NaOCl at 4°C
×277 magnification.

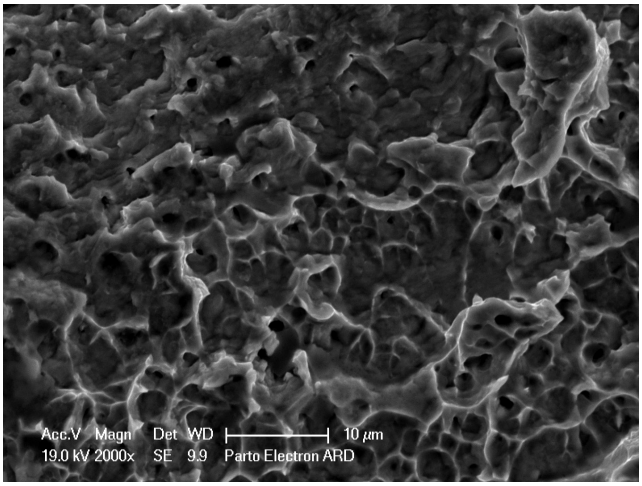

Fig. 5. Scanning electron microscopy (SEM) image of the ProTaper Gold (PTG) S1 instrument after cyclic fatigue testing using NaOCl at 22°C
×2,000 magnification.

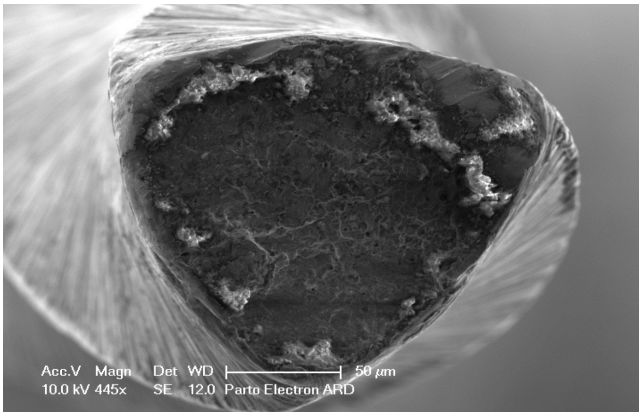

Fig. 6. Scanning electron microscopy (SEM) image of the ProTaper Gold (PTG) S1 instrument after cyclic fatigue testing using NaOCl at 37°C
×445 magnification.

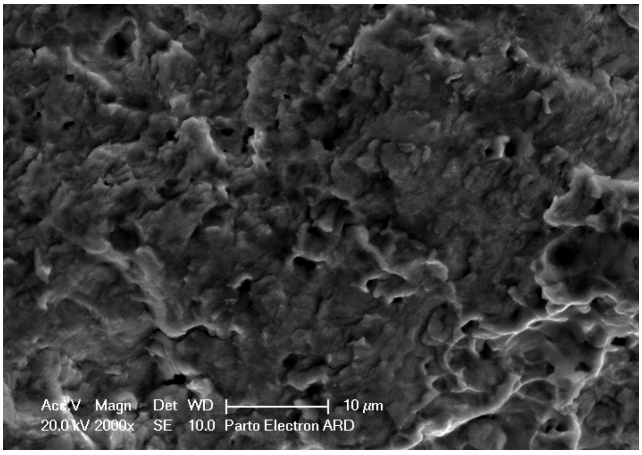

Fig. 7. Scanning electron microscopy (SEM) image of the ProTaper Gold (PTG) S1 instrument after cyclic fatigue testing using NaOCl at 37°C
×2,000 magnification.

Results

The *M* and *SD* values for NFC are presented in Table 1. The mean fracture resistance in group 1 was slightly higher than that in the other groups. The mean fracture resistance in group 2 was slightly lower than that in the other 2 groups; however, there were no significant differences in the mean NCF values between the 3 groups ($p > 0.05$). Table 1 presents the mean length of the broken pieces at 22°C, 4°C and 37°C; there was no statistically significant difference between the 3 groups in this respect ($p > 0.05$).

Table 1. Number of cycles to fracture (NCF) and length of the broken pieces in all study groups

Group	NCF	Length of the fractured piece [mm]
Group 1	1248.50000 ±299.17526	4.42 ± 0.45
Group 2	1076.50000 ±190.93411	4.98 ± 0.56
Group 3	1119.50000 ±117.81174	4.48 ± 0.71

Data presented as mean ± standard deviation (*M* ±*SD*).
Group 1 – NaOCl at 22°C; group 2 – NaOCl at 4°C; group 3 – NaOCl at 37°C.

Discussion

The introduction of rotary files has resulted in faster root canal therapy and fewer procedural errors during root canal preparation. One of the most common errors during root canal therapy is file fracture within the root canal. The highest probability of fracture is related to fatigue. Factors that affect fatigue resistance include the canal curvature, the length and anatomy of the root canal, the frequency of file use, the design of the file, the metal alloys used in the file, the sterilization process, the rotary machine factors, and the operator's experience.[15–18]

Recently, the effect of environmental temperature has been investigated as a factor affecting fracture resistance.[10,19] The majority of these studies have investigated the effect of temperatures higher than the temperature within the root canal. A temperature of 4°C is considered a low temperature of the sodium hypochlorite solution; also, the sodium hypochlorite solution is stable at this temperature.[20] Temperatures of 37°C and 22°C are considered root canal and environmental temperatures, respectively. A concentration of 5.25% was selected for the sodium hypochlorite solution in this study, since it is commonly used in root canal therapy.[21]

In this study, fatigue resistance was determined with a device that was similar to that used in previous studies.[1,8,22] The length of the broken pieces was not significantly different between the groups, which indicates the presence of a similar location of stress in the root canal, i.e., the middle of the curvature in the root canal, which is consistent with previous studies.[10,22]

Previous clinical studies have shown that when the irrigation solution is delivered into the root canal at different temperatures, the body tends to balance its temperature with the temperature within the canal, which is around 35°C.[21] One of the factors that can influence fracture resistance is the temperature at conversion from the austenitic phase to the martensitic phase during the fabrication of the file alloy. If this temperature is between room temperature and the temperature within the root canal, the file temperature reaches the phase transition temperature when the file is inserted into the canal, resulting in a decrease in fatigue resistance and an increase in the odds of fracture.[23]

One of the advantages of PTG Gold files over previous generations is that its phase transition temperature is higher than the temperature within the root canal.[23] In this study, unlike in previous studies, the temperature of the solution did not affect the fracture resistance of the files, which might be attributed to the phase transition temperature; therefore, in heat-treated files, temperature has a lesser effect on the fracture resistance of the files.[24]

In another study, the effect of 3 temperatures of the sodium hypochlorite solution (22°C, 37°C and 50°C) on the fracture resistance of files was investigated.[14] It was reported that a temperature of 37°C had no effect on fracture resistance, which is consistent with the results of the present study, while a temperature of 50°C increased the fracture

resistance of the rotary files.[14] Another study determined that a decrease in environmental temperature to 0°C increased the fracture resistance of the tested files.[19]

Previous studies have shown that contact between the sodium hypochlorite solution and Ni-Ti files might result in the corrosion of the files. If this corrosion does not occur in the area of file which is affected by high stress, it cannot affect the fracture resistance of the file.[9] According to the manufacturer's claims, in newer generations, files do not undergo corrosion due to more advanced metallurgy. For example, a study on the effect of high concentrations of the sodium hypochlorite solution at 22°C, 37°C and 50°C determined that the concentration of the sodium hypochlorite solution did not affect the fracture resistance of rotary files, which is consistent with the results of the present study.[4]

A similar study compared the effects of environmental temperature and the temperature within the root canal on the fracture resistance of ProTaper Universal and PTG files.[23] It concluded that temperature did not affect the fracture resistance of PTG files, while increasing the temperature decreased the fracture resistance of ProTaper Universal files, which is consistent with the results of the present study.[23]

Since the fracture resistance of files depends on different factors, including the type of file and the method applied in the study, it is suggested that other files and temperatures should also be evaluated. In addition, since this study was carried out in vitro, it is suggested that, if possible, clinical studies should be carried out on vital teeth.

Conclusions

Increasing the temperature of the sodium hypochlorite solution to 37°C or decreasing it to 4°C did not significantly affect the fracture resistance of PTG rotary files. In addition, the length of the broken pieces was not significantly different between the 3 groups. Since this study was carried out in vitro, it is suggested that further clinical studies be undertaken on vital teeth.

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Impact of the COVID-19 pandemic on the dental service: A narrative review

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Abstract

This article describes what changes have had to be made to the functioning of dental practices due to the COVID-19 pandemic and how the pandemic has affected dental staff. Dentists are at high risk of infection and this is associated with fear, including the fear of being infected by their co-workers and patients, or that they will infect their families. The introduced changes include increased protective measures, and the introduction of additional questionnaires and procedures. In dental practices, the use of personal protective equipment (PPE) has been increased and changes have been introduced in the functioning of surgeries in accordance with the recommendations of dental associations and governments. The aforementioned changes have significantly reduced the comfort of dental work, increased the costs of treatment and reduced the availability of dental treatment. A novel solution to this situation has been the implementation of teledentistry, which helps to reduce the number of non-emergency visits. This process involves the remote facilitation of dental treatment by means of technology (i.e., phone or the Internet) without direct contact with the patient. Due to the restrictions implemented during the pandemic, many universities have introduced remote or hybrid teaching for both didactic and practical classes.

Keywords: dentistry, dental practice, dental education, COVID-19, pandemic

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the 7th virus in the coronavirus family. This virus is very easily transmitted by contact with infected individuals, both symptomatic and asymptomatic.[1] Restricting out-of-home movement, social distancing, the cessation of almost all work activities, and wearing protective masks and gloves are intended to minimize the transmission coronavirus disease 2019 (COVID-19).[2] The COVID-19 pandemic has had a significant impact on the lives of all human beings.[3,4] Contact with other people has been reduced or eliminated, which has significantly affected mental health along with personal and professional life.[5,6] Medical professions, depending on their specialization (different distances between workers and patients), are exposed to infectious agents to varying degree.[7] Dentists are most at risk of COVID-19 infection, even more than nurses and general practitioners.[2] This fact results from immediate contact with the oral cavity, where the virus is present. Moreover, water spray, which can reach up to 2–3 m from the patient's mouth, considerably increases dental personnel's exposure to the virus.[8,9] This has resulted in many changes in the functioning of dental practices.[10,11]

The aim of this article was to describe and discuss changes in the availability and quality of dental services and dental education during the COVID-19 pandemic based on a review of the literature.

Method

The selected articles were obtained by searches, using Google Scholar and PubMed. The selection of scientifically valid sources took place between December 1 and 29, 2020. The following keywords were used: 'COVID-19'; 'SARS-CoV2'; 'pandemic'; 'coronavirus'; 'dentistry in the pandemic'; 'teledentistry'; 'dental education'; 'stress'; 'protective measures in the pandemic'; 'psychological impact'; and 'mental health'. Out of 96 retrieved articles, 51 items were selected for this paper. Bibliographic reviews, systematic reviews, meta-analyses, cohort studies, and studies in English, Polish or Croatian were included. The exclusion criteria were as follows: articles not related to the topic; animal studies; full text not available; and articles in other languages. No time limits were applied during the screening phase with regard to the scientific articles.

Psychological consequences of COVID-19

At the onset of the pandemic, there was no knowledge on the transmission of SARS-CoV-2, and therefore no guidelines were introduced. As a result, dentists did not fully know how to proceed, and the first reaction of many of them was to close down their offices at the end of March 2020.[12]

Doctors' main concerns were the fear of infecting their family, their own safety and the awareness of the mortality due to COVID-19 infection.[13,14] Healthcare workers were not immune to the psychological consequences of COVID-19.[15] They were at higher risk of developing anxiety, depression and high levels of stress during the outbreak of SARS-CoV-2.[15,16] The reasons for this included excessive workload, inadequate personal protective equipment (PPE) and the feeling of being inadequately supported.[15,17]

The concern about being infected with COVID-19 by a patient was a major psychological strain.[14,18] Furthermore, psychological distress has been identified as a cause of the potential reduction in the quality of treatment provided. It seems that young practitioners became more stressed when the spread of COVID-19 accelerated. It was noted that decision-making skills, clinical experience, the ability to deal with the encountered difficulties, and job satisfaction were higher in specialized dentists, who managed stress more effectively than novices.[19]the aim of this study was to evaluate the level of perceived stress (PS Moreover, dentists working within the private sector (clinics and/or institutions) experienced higher levels of stress than dentists working in hospitals or state institutions.[19]

A survey conducted in March 2020 among 650 dentists from 30 countries in Europe, Asia, Australia, and North America showed that 66% of respondents wanted to close their practices until a significant decrease in COVID-19 cases, 87% of individuals were afraid of being infected, 92% had concerns about infecting family members, and 72% felt nervous talking to a patient in close proximity.[10] Moreover, a survey conducted in April 2020 among 365 dentists in Northern Italy reported that 6.2% of respondents experienced anxiety intensely, 37.4% experienced it lightly and 23.6% experienced it moderately.[20] Intense sadness was felt by 12.6% of individuals and intense anger by 9.3%, while 44.1% of respondents did not experience anger at all.[20] In conclusion, health professionals experienced different emotions and moods at different levels, but anxiety and stress were the most common. The fear of infection was inherent in the experiences of dental professionals.

Changes in the organization of dental practices

In the dental practices which were reopened or were not previously closed, increased personal protection measures and changes in the functioning of these practic-

es were applied in accordance with the rapidly changing recommendations from dental associations and governments.[8,21] Dentists were to avoid planned patient treatment during the COVID-19 pandemic, with only pain patients being treated and those needing urgent assistance.[2,21] The recommendations also included abstaining from the aerosol-generating procedures which involve using a turbine, a handpiece or air-water syringes. Unfortunately, these instruments are necessary to achieve the right quality of treatment.[22]

Changes in the functioning of dental practices included larger intervals between patients and fewer patients being treated to prevent contact in the waiting room, increased frequency of disinfection, taking additional medical history of the potential contact with a coronavirus patient or COVID-19 symptoms, and more restrictive treatment protocols.[8,21] The SARS-CoV-2 virus can persist on surfaces from a few hours to several days, depending on the type of surface, temperature or humidity. This reinforced the need for thorough disinfection of all surfaces in the dental practice.[23] Each area in the waiting room was associated with a risk of infection. Therefore, in addition to ensuring adequate periodic air exchange, all surfaces, chairs, magazines, and doors that could come into contact with medical staff and patients were to be treated as "potentially contaminated".[2] The complete removal of leaflets and magazines from the dental office was also to be considered.[24] In order to reduce the number of people in the office, people accompanying the patient were asked to wait outside or in a car, the exception being when the patients were unable to arrive to the appointment by themselves due to their health condition.[2] In the waiting room, the chairs were taped and marked with social distance signs.[24] The whole air conditioning system required strict and frequent decontamination.[2]

Protective measures

Personal protective equipment included N95 or preferably N99 masks (FFP2 and FFP3, respectively), disposable aprons and caps, easily washable shoes, and additional protection of the doctor's eyes and face in the form of a face shield, instead of just protective glasses.[8] Proper hand hygiene has been identified as the most important factor in reducing the transmission of microorganisms to patients.[23] Dentists were advised to avoid or minimize situations that may cause the formation of aerosol. The use of high-volume saliva ejectors was advised to reduce the production of droplets and aerosol.[2] Since the number of pathogens contained in the human saliva is very high, rinsing the mouth with antiseptic liquids can reduce the amount of infectious particles, but cannot eliminate the virus from the saliva entirely.[2] Many products, such as chlorhexidine (CHX), cetylpyridinium chloride (CPC) and essential oils (EO), have been used in oral rinses. They exhibit antibacterial, antifungal and antiviral properties.[25] The use of a rubber dam while performing procedures was recommended to reduce contact with the saliva by reducing the number of droplets around the operating field by 70%.[8] If the use of a rubber dam was not possible, it was recommended to treat the cavities with hand tools.[8] The use of a face shield was highly recommended. However, the use of magnifying glasses and a microscope was cumbersome due to the bulk of the mandatory attire.[8] Minimally invasive procedures were also recommended to avoid increased salivary secretion, coughing or vomiting, which also may affect the quality of treatment.[8] The procedures involving taking tomography or extraoral X-rays instead of intraoral projections were favored. Moreover, the use of scaling or other treatment producing spray needed to be limited.[8,12,21]

In addition, the time required for the treatment of a single patient has been prolonged, due to the initial interviewing of patients for COVID-19-related medical history, pre-qualification surveys for COVID-19 and the measurement of body temperature.[26]

Cost increases

The aforementioned changes have significantly reduced the comfort of dental work, increased the costs of treatment and reduced the availability of dental services.[21] Financial stress and anxiety are some of the important aspects of financial mental health, which can impact an individual's cognitive, emotional and relational well-being.[19] According to a telephone survey carried out between March 24 and April 2, 2020 in Germany, carried out after 135 days of restrictions, 12–29% of practices were not able to cover their operating costs.[27] The longer the restrictions, and the associated closures, reductions in the number of patients and increases in the amount of new PPE last, the worse off dental practices and clinics will be.[27]

The overall need to introduce the aforementioned additional PPE came with additional costs. Since the start of the pandemic, the costs of personal protective measures and additional disinfectants have increased.[21] As the number of patients has been reduced and costs have grown, some dentists have been forced to introduce additional fees for each visit or to increase the price of treatment.[26] A partial economic evaluation was carried out in Brazil, using the activity-based costing method to calculate the purchase of the PPE and decontamination solutions recommended for dental clinical practices during the COVID-19 pandemic.[28] Two scenarios were compared – pre-COVID-19 and post-COVID-19, by taking prices from at least 3 online quotations made in May 2020. The pre-COVID-19 scenario included the standard use of disposable gloves, disposable masks, disposable caps, disposable coats, and safety glasses. The post-COVID-19

scenario included the use of disposable gloves, N95/FFP2 masks, disposable masks, disposable caps, disposable shoe covers, waterproof medical coats, disposable gowns, protective goggles, and face shields. Each disposable item was replaced after each patient. The waterproof medical coat was recommended to be used for the whole day. The dentists and dental hygienists were advised to use protective goggles and face shields, which were disinfected between patients. The cost of the protective goggles and face shields was spread over the number of uses and the equipment was recommended to be replaced every 6 months. Prior to the COVID-19 pandemic, the direct costs of biosafety recommendations amounted to R$0.84 per patient, R$6.69 per service shift and R$3,413.94 per year. The post-COVID-19 costs of biosafety recommendations resulted in R$16.01 per patient, R$128.07 per service shift and R$32,657.96 per year. The costs of disposable PPE in the post-COVID-19 scenario consisted of R$122, which was 95.26% of the total.[28] The results of the abovementioned study show that changes in biosafety protocols during the COVID-19 pandemic have significantly increased the costs of dental consultations.[28] Most fixed costs, such as the staff costs, materials and installments, are difficult to reduce and require redundancies.[27] A large number of costs, such as the costs of disinfectants, PPE and disposable materials, will only grow.[20,27] Without increases in the service costs, dental clinics will not be able to survive.[27] However, an increase in the treatment costs means a decrease in the availability of dental services, and, while almost all large- and small-scale sectors are on the verge of losing their ground, the dental healthcare sector is no exception.[1,19,27]

Teledentistry

The closure of dental offices resulted in the lack of proper dental care, and consequently a deterioration in patients' oral health.[12] However, a new and interesting solution to the situation of the pandemic turned out to be teledentistry. Teledentistry involves the remote facilitation of dental treatment by means of technology (i.e., phone or the Internet) without direct contact with the patient.[29] It can take the form of a real-time consultation or be realized through the store-and-forward method.[30,31] A real-time consultation involves a video conference between the doctor and the patient.[32] Based on the patient's history and clinical symptoms, the dentist makes a decision about the necessity of the patient's appearance at the dentist's office. In situations where dental treatment does not have to be undertaken immediately, appropriate detailed instructions for home medical care should be provided by means of teleinformation and prescriptions should be issued for the recommended medication.[1] Additionally, the store-and-forward method involves sharing clinical information between specialists for consultation and treatment planning.[33] The patient does not participate in this form of consultation.[29,34] There may be exchanges between physicians, involving radiographs, graphical representations of periodontal and hard tissues, treatment, laboratory results, photographs, and other information transportable through multiple providers.[34] A third method has also been described, known as the remote monitoring method, in which patients are monitored remotely in real time, and can be either hospital-based or home-based.[35]

Teledentistry has the potential to address patients' therapeutic needs and reduce healthcare costs.[34] It enables redressing the balance in access to dental care for patients in rural areas, nursing homes, and those who have mobility or transportation problems.[1,31,36] Moreover, it plays an important role in many fields of dentistry and enables rapid diagnoses of patients at a distance. In the case of impacted or semi-impacted third molars, a remote diagnosis brings the same results as a real-time diagnosis.[37] With the help of teledentistry, orthodontic specialists can prepare, advise, and even supervise general dentists when a patient is unable to see an orthodontist.[38] A diagnosis of caries in young children is performed just as well with teledentistry as it is conventionally.[39] Of course, it will not replace most dental visits, but at the same time provides many advantages.[30]

Education of future dentists

Medical and dental universities educating future doctors and dentists are also facing a problem. Many universities have introduced online or hybrid teaching. Social distancing continues to be one of the most effective means of protection against the infection. Therefore, classes with a larger number of students, such as lectures and seminars, have been moved completely to online platforms.[40,41] All types of knowledge testing, such as exams, colloquia and tests, have been conducted online as well. Whether or not such solutions can produce measurable effects on education will have to be evaluated in the future.[40]

Although e-learning is considered enjoyable by many students,[42,43] they are still worried about acquiring practical skills entirely through online learning.[44] Some dental students may have fewer opportunities to practice and develop their manual and practical skills, which are particularly important in dentistry.[45] Learning on phantoms during pre-clinical classes can only partially compensate for the lack of contact with actual patients.[46]

The fact that students might not learn certain practical skills due to the lack of actual clinical classes could prove to be a major problem. The consequence of this could be an extended transition time from a student to a qualified healthcare professional, due to the need to catch up on their clinical skills. Students of senior years are faced with

the worst situation, since they will not have time to catch up, in contrast to younger students. Therefore, many universities have prioritized the organization of classes for students who perform clinical procedures.[47] Many students have therefore expressed the need to return to the traditional form of education in the near future. However, they realize that the way of education may have changed for the years to come.[48] On the other hand, a positive effect of online education turned out to be that it has encouraged students to expand their knowledge on their own and to use resources provided on the Internet, such as webinars, clinical videos, etc.[48,49]

Changes in the way education is provided have regarded not only students, but also academic teachers, who additionally do clinical work in their own offices. The changes caused by the pandemic have significantly affected both areas of their responsibilities. New solutions and procedures, which continue to be introduced practically overnight, result in increased feelings of stress and anxiety. Doctors who are also academic teachers speak out about the burden they feel particularly during this time of crisis.[47,50]

Overview and future perspectives

During the course of the pandemic, increased feelings of fear, anxiety and sadness have been observed among dentists. Due to these emotions, many practices closed down at the beginning of the pandemic, making it difficult for patients to access treatment. Changes such as extended breaks between patients and accepting only urgent cases had a similar effect. Increased safety measures, such as additional protective clothing, antiseptics, etc., resulted in increased treatment costs. Increases in prices were not always reimbursed. Choosing alternative methods of treatment may not give equally good results and could result in a lower quality of dental services. However, the full quality of dental treatment during the COVID-19 pandemic will certainly still be examined from a long-term perspective.

Based on the literature, it can be concluded that the longer the pandemic lasts, the higher the stress among medical workers. Personal protective equipment will become part of the daily operation of dental practices. Teledentistry will develop more rapidly and on a larger scale, so that it can be of use to patients more than it used to be. Modern technology and wide access to the Internet could make this possible. The Internet will also play a greater role in the education of future dentists. However, it will not replace practical classes and contact with real patients.

To sum up, the COVID-19 pandemic is forcing changes in the education of dental students as well as in everyday dental practice. It also affects the availability and quality of dental services for patients. Consequently, new procedures have been developed and quickly implemented into everyday work. Until the epidemiological situation calms down, the changes introduced will stay with us longer and could possibly last forever.

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Recognition and treatment of peri-implant mucositis: Do we have the right perception? A structured review

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Abstract

Peri-implant mucositis is a common inflammatory lesion of the soft tissues surrounding endosseous implants, with no loss of the supporting bone. Its prevention or early diagnosis are vital for dental implant success.

The aim of this review was to investigate knowledge strengths and gaps in clinicians' perceptions of peri-implant mucositis prevalence and evidence for successful treatment.

A literature search for articles published until 2020, reporting on the prevalence of peri-implant mucositis and its treatment was performed in standard online databases. The inclusion criteria were as follows: studies in English; studies with an available abstract; studies on humans with at least 1 dental implant; and studies reporting on the prevalence and/or treatment of peri-implant mucositis. Sixty-five studies fulfilled the inclusion criteria. The included papers were analyzed to identify data on the prevalence and treatment of peri-implant mucositis. The prevalence statistics for peri-implant mucositis had wide ranges in both the patient-based (PB) analysis and the implant-based (IB) analysis; the possible reasons for these wide ranges are discussed. Treatment methods for peri-implant mucositis were analyzed individually and compared to the management of gingivitis.

It was determined that the currently available information on the prevalence rates and the standardized therapeutic protocols for peri-implant mucositis are insufficient. Since the mean gingivitis and peri-implant mucositis prevalence rates in the PB analysis were similar, it is possible that peri-implant mucositis is underestimated due to variables related to implant rehabilitation itself.

Keywords: inflammation, dental implant, literature review, oral mucositis, peri-implant healing

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Introduction

Dental implants are widely used for oral rehabilitation. They are biocompatible prosthetic devices implanted in living bone and, for this reason, the peri-implant tissue conditions can change over time.[1,2] Healthy peri-implant tissues are characterized by the absence of erythema, bleeding on probing (BoP), swelling, and suppuration.[3]

Once osteointegration has been achieved, allowing for the healing time after implant insertion, implant complications can occur due to mechanical problems, inflammation and/or the loss of the surrounding tissues (the oral mucosa and the supporting bone). These could lead to relevant discomfort for the patient as well as implant failure over time.[4] After osteointegration has occurred, implants may become contaminated and peri-implant tissues could become inflamed, causing peri-implant mucositis and/or peri-implantitis.[3]

In an animal study on beagle dogs, Berglundh et al. compared the anatomy and histology of peri-implant and periodontal tissues in block biopsies.[5] A histological examination showed that both presented well-keratinized areas (the oral epithelium and the outward portion of the peri-implant mucosa), but periodontal tissues presented only a few cells of thick epithelium in contact with the implant abutment. Also, peri-implant tissue fibers displayed a parallel course originating from the crestal bone, while periodontal tissue fibers were perpendicular to the dental root, going from the root cementum to the alveolar bone.[5]

Likewise, blood supply differed from an anatomical point of view – the peri-implant bone vasculature consisted only in the periosteum source, while gingival supply was guaranteed by a double source composed of supraperiosteal and periodontal ligament vessels.[6]

Being aware of histological differences between peri-implant tissues and the periodontium is fundamental to better understand the peri-implant tissue biology. Clinicians, implant-rehabilitated patients and the dental industry have mainly based their maintenance approaches on the techniques and tools derived from the pre-implant era.

Peri-implant mucositis is an inflammatory lesion of the soft tissues surrounding an endosseous implant, with no loss of the supporting bone or the continuing marginal bone.[7] Conversely, peri-implantitis is described as a pathological condition occurring in tissues around dental implants that is characterized by inflammation in the peri-implant connective tissue and a progressive loss of the supporting bone.[8]

The etiology of peri-implant mucositis has been described as the accumulation of bacterial biofilm around the implant, which may cause signs and symptoms of inflammation, such as local swelling, redness, pain, and BoP.[7] The diagnosis of peri-implant mucositis vs. peri-implantitis is made by the evidence of pathological bone loss.[9] While peri-implant mucositis exhibits signs of inflammation with no bone loss besides the remodeling process of the alveolar bone during the first year after implantation, peri-implantitis shows signs of inflammation associated with a further loss of the crestal bone.[3,7] In recent years, there has been a general consensus that following the first year of implant functioning, bone loss around dental implants ≥2 mm represents peri-implantitis.[10]

Data indicates that patients diagnosed with peri-implant mucositis may develop peri-implantitis, especially in the absence of regular maintenance care, but the processes and reasons for this pathological progress remain unknown.[11] Factors associated with peri-implant mucositis include biofilm accumulation, smoking and radiation therapy.[7] Regular supportive peri-implant care with biofilm removal is an important preventive strategy against the conversion of a healthy tissue to peri-implant mucositis, and also against the progression of peri-implant mucositis into peri-implantitis.[7,11]

There is evidence that peri-implant mucositis can be successfully treated. The resolution of the clinical signs of inflammation may take more than 3 weeks following the restoration of plaque/biofilm control.[11] The management of peri-implant inflammation should be addressed in terms of infection control, decontamination of the implant surface and regeneration of the alveolar bone when needed.[12]

The early diagnosis and prevention of peri-implant infections are essential for the long-term dental implant success. In order to perform a thorough evaluation of the peri-implant conditions, peri-implant probing and relative radiographs are always required.[12,13]

The purpose of this review was to highlight possible clinicians' perception problems related to peri-implant mucositis, to investigate the prevalence of peri-implant mucositis reported in the literature and to analyze the evidence-based data regarding its treatment.

Material and methods

Focus question

The focus question for the literature search was: "What is the clinician's perception regarding the prevalence levels and treatment strategy efficacy/evidence for peri-implant mucositis?"

It was structured according to the PICO format[14]:

– Population: patients rehabilitated with dental implants;
– Intervention: implant prosthesis, peri-implant tissue, and peri-implant mucositis prevalence and treatment;
– Comparison: diagnostic criteria and peri-implant mucositis treatment;
– Outcome: finding consistency between prevalence and perception, and differences between various kinds of treatment.

Search strategy

The PubMed/MEDLINE, Embase, Scopus, Web of Science, and Cochrane databases were searched to identify published articles reflecting the inclusion criteria: studies in English; studies with an available abstract; studies involving humans with at least 1 dental implant; and studies reporting data on the prevalence and/or treatment of peri-implant mucositis. The search strategy was divided into 2 parts: a pre-search to avoid discrepancies between findings due to the device used (a personal computer or a mobile device); and a focus question search.

The pre-search was used to determine the device and keywords that provided the greatest number of results in order to establish the focus question search. The pre-search concerned peri-implant mucositis studies published up to 2020. The terms used for the identification of keywords were: 'peri implant' OR 'peri-implant' OR 'peri-implant mucositis' AND 'mucositis'.

The focus question search was carried out on a personal computer to analyze the abovementioned databases, using the 2 keywords that yielded the greatest number of results in the pre-search. The focus question search concerned peri-implant mucositis studies published up to 2020. The terms used for the identification of keywords were: 'peri implant mucositis' OR 'peri-implant mucositis' AND 'prevalence' OR 'treatment'.

The focus question search yielded 99 articles for "peri implant mucositis prevalence", 99 for "peri-implant mucositis prevalence", 300 for "peri implant mucositis treatment", and 271 for "peri-implant mucositis treatment".

Screening and selection

The inclusion criteria were as follows: studies in English; studies with an available abstract; studies involving humans with at least 1 dental implant; and studies reporting on the prevalence and/or treatment of peri-implant mucositis.

The exclusion criteria were as follows: studies in a language other than English; studies without an available abstract; non-clinical studies; studies without dental implants; and studies reporting on neither the prevalence nor the treatment of peri-implant mucositis.

Once the studies were selected according to the abovementioned initial screening, only those fitting the following categories were included: randomized clinical trials (RCTs); controlled clinical trials (CCTs); cohort studies; cross-sectional studies; and case–control studies.

The studies were first screened by titles and abstracts, and examined by 2 reviewers. The full text of the selected articles was retrieved and the study results were analyzed. Review articles and systematic reviews were also studied in order to find other articles that did not emerge during database inquiries.

Full-text studies admitted for final analysis were divided into 2 groups: the prevalence group; and the treatment group.

Results

Sixty-five studies fulfilled all the inclusion criteria: 25 RCTs; 3 CCTs; 15 cohort studies; 20 cross-sectional studies; and 2 case–control studies. All these studies were divided into 2 main groups according to the 'prevalence' (n = 34) or 'treatment' (n = 31) Medical Subject Headings (MeSH). The results according to the type of study are shown in Table 1 for the prevalence group and in Table 2 for the treatment group.

In the prevalence group, cohort and cross-sectional studies constituted the majority of the research devoted to peri-implant mucositis (Table 1). In cohort studies, the peri-implant mucositis prevalence rates ranged between 7.14% and 68.00% in the patient-based (PB) analysis (referring to the number of patients included in the analysis), and between 5.06% and 38.00% in the implant-based (IB) analysis (referring to the number of implants included in the analysis). In cross-sectional studies, the peri-implant mucositis prevalence ranges varied from 20.80% to 80.90% in the PB analysis, and from 21.00% to 90.00% in the IB analysis (Table 3).

In the treatment group, there were RCTs, CCTs, cohort studies, and 1 case–control study (Table 2). The search found 1 RCT on the use of sodium hypochlorite gel, 1 RCT about the modification of the prosthesis, 1 RCT on the use of a drying agent associated with manual debridement, 2 RCTs in which chlorhexidine gel was used, 1 RCT

Table 1. Prevalence group results according to the type of study

Type of study	Number of articles
RCTs	0
CCTs	0
Cohort studies	13
Cross-sectional studies	20
Case–control studies	1

RCT – randomized clinical trial; CCT – controlled clinical trial.

Table 2. Treatment group results according to the type of study

Type of study	Number of articles
RCTs	25
CCTs	3
Cohort studies	2
Cross-sectional studies	0
Case–control studies	1

Table 3. Peri-implant mucositis prevalence ranges according to the type of study

Type of study	Prevalence range [%]	
	PB analysis	IB analysis
Cohort studies	7.14–68.00	5.06–38.00
Cross-sectional studies	20.80–80.90	21.00–90.00

PB – patient-based; IB – implant-based.

Table 4. Treatment proposed with regard to the type of study

Type of study	Treatment tested
RCTs, CCTs and a case–control study	sodium hypochlorite gel
	modifying the prosthesis
	desiccant agent
	chlorhexidine gluconate
	cetylpyridinium
	triclosan
	chitosan brushes
	probiotics
	diode laser
	photodynamic therapy
	air polishing
	enamel matrix derivative
	ozone
	hydrogen peroxide
	systemic antibiotics
	azithromycin
	mechanical curettage
Cohort studies	non-surgical therapy

in which a mouth rinse with 0.03% chlorhexidine and 0.05% cetylpyridinium was assessed, 1 RCT that investigated 0.12% chlorhexidine gluconate, 3 RCTs in which toothpastes containing triclosan were assessed, 1 RCT in which chitosan brushes were used, 5 RCTs about probiotics (in one of the studies, photodynamic therapy was added to probiotic administration), 2 RCTs about photodynamic therapy, 3 RCTs about air polishing, 1 RCT in which an enamel matrix derivative was used, 1 RCT on the use of ozone and/or hydrogen peroxide, and 2 RCTs in which systemic antibiotics supported mechanical debridement. The 2 cohort studies were about mechanical debridement and biofilm control (Table 4).

The selected studies proposed various kinds of treatment, including sodium hypochlorite gel, a desiccant agent, chlorhexidine, triclosan, chitosan brushes, probiotics, diode laser therapy, photodynamic therapy, air polishing, and antibiotics. Most of these consisted of mechanical debridement combined with an additional therapy, such as sodium hypochlorite gel, a desiccant agent, chlorhexidine, probiotics, photodynamic therapy, an enamel matrix derivative, and systemic azithromycin (Table 5).

Table 5. Treatment proposed and related results and conclusions

Treatment	Authors, year of publication	Study type	Study description	Sample size	Implant number	Results	Conclusions
Sodium hypochlorite gel	Iorio-Siciliano et al. 2020[28]	triple-blind RCT 6-month follow-up	mechanical debridement with sodium hypochlorite gel (test group) vs. mechanical debridement with placebo gel (control group)	46	68	PPD decreased in both the test and control groups ($p = 0.0001$ and $p = 0.0001$, respectively)	a complete resolution was not achieved with either therapy
Modifying the implant-supported prosthesis	de Tapia et al. 2019[24]	RCT 6-month follow-up	modifying the prosthesis to allow better oral hygiene (test group) or not (control group)	45 test – 24 control – 21	145	changes in mBI in the test and control groups were 1.14 and 0.50, respectively ($p = 0.010$), in PPD – 0.31 mm and 0.02 mm, respectively ($p = 0.040$)	modifying the prosthesis improved clinical outcomes
Topical desiccant agent in association with manual debridement	Lombardo et al. 2019[29]	RCT	desiccant agent after debridement (test group) vs. 1% chlorhexidine after debridement (control group)	23	52	the test group presented significantly greater reductions in BoP, mBI, VPI, and mPI than the control group	a complete resolution of the inflammatory conditions was not achieved by either group
Chlorhexidine-containing brush-on gel	Hallström et al. 2017[42]	double-blind RCT 12-week follow-up	chlorhexidine-containing brush-on gel used as an adjuvant to mechanical debridement	37	37	the test group presented a reduction in BoP after 4 and 12 weeks as compared to the control group ($p < 0.05$)	the findings indicate moderate but significant improvement in clinical parameters
Chlorhexidine gel	Heitz-Mayfield et al. 2011[46]	RCT	non-surgical debridement with/without 0.5% chlorhexidine gel	29 test – 15 control – 14	29	at 1 month and from 1 to 3 months, there were statistically significant reductions in the mean number of sites with BoP and the mean PPD values at implants in both groups	adjunctive chlorhexidine gel did not improve the results as compared to mechanical cleaning alone

Treatment	Authors, year of publication	Study type	Study description	Sample size	Implant number	Results	Conclusions
0.03% chlorhexidine and 0.05% cetylpyridinium mouth rinse	Pulcini et al. 2019[30]	double-blind RCT 12-month follow-up	0.03% chlorhexidine and 0.05% cetylpyridinium mouth rinse vs. placebo mouth rinse	46 test – 24 control – 22	54	a reduction in BoP in the test group ($p = 0.002$) and the control group ($p > 0.05$)	the use of the test mouth rinse demonstrated some adjunctive benefits in peri-implant mucositis treatment
0.12% chlorhexidine gluconate	Menezes et al. 2016[39]	RCT 6-month follow-up	basic periodontal therapy with 0.12% chlorhexidine gluconate mouthwash vs. basic periodontal therapy and placebo mouthwash	37	119 test – 61 control – 58	significant improvement in comparison with baseline, no significant differences between the treatment groups	0.12% chlorhexidine was not more effective than placebo
Triclosan dentifrice	Ramberg et al. 2009[26]	double-blind RCT 6-month follow-up	dentifrice containing triclosan vs. sodium fluoride dentifrice	60	N/A	subjects with peri-implant mucositis who used a 0.3% triclosan dentifrice exhibited significantly fewer clinical signs of inflammation than subjects who used a regular fluoride dentifrice	the regular use of triclosan dentifrice may reduce the clinical signs of inflammation
Triclosan-containing fluoride toothpaste	Pimentel et al. 2019[31]	RCT two 3-week follow-ups	triclosan/ fluoride toothpaste vs. fluoride toothpaste	26	N/A	both groups showed increases in PI ($p = 0.001$)	triclosan-containing toothpaste reduced the RANKL/OPG ratio
Triclosan-containing toothpaste	Ribeiro et al. 2018[35]	RCT 6-week follow-up	triclosan/ copolymer/ fluoride toothpaste vs. placebo fluoride toothpaste	22	22	both groups showed increases in PI at implant sites from the 3rd to the 21st day, avoiding an increase in BoP throughout the follow-up was possible only with triclosan treatment	triclosan-containing toothpaste controls the clinical signs of inflammation
Chitosan brush	Wohlfahrt et al. 2019[25]	RCT 6-month follow-up	chitosan brush on an oscillating dental handpiece vs. titanium curette	11	24	both groups demonstrated significant reductions in BoP between baseline and 6 months	a chitosan brush seems to be a safe and efficient device for the debridement of dental implants
Probiotics	Galofré et al. 2018[20]	triple-blind RCT	oral probiotic *L. reuteri* as an adjuvant to non-surgical mechanical therapy	44 with peri-implant mucositis – 22 with peri-implantitis – 22	44	a decrease of *P. gingivalis* bacterial load at implant sites with mucositis ($p = 0.031$)	the probiotic together with mechanical therapy produced additional improvement over treatment with mechanical therapy alone
Probiotics	Peña et al. 2019[32]	triple-blind RCT 3-month follow-up	mechanical debridement with 0.12% chlorhexidine and *L. reuteri* vs. mechanical debridement with 0.12% chlorhexidine	50	50	after the administration of 0.12% chlorhexidine, all clinical parameters improved in both groups	the administration of the probiotic did not seem to provide an additional clinical benefit
Probiotics	Hallström et al. 2016[43]	double-blind RCT 26-week follow-up	probiotic supplements as an adjuvant to conventional management vs. placebo	49	N/A	after 4 and 12 weeks, BoP and PPD significantly decreased in both groups ($p < 0.05$), no significant differences between the treatment groups	probiotic supplements did not provide additional improvement over placebo

Treatment	Authors, year of publication	Study type	Study description	Sample size	Implant number	Results	Conclusions
Probiotics	Flichy-Fernàndez et al. 2015[44]	double-blind RCT	*L. reuteri*	34	77	after treatment with the probiotic, patients with mucositis and without peri-implant disease showed improvement in clinical parameters, with reductions in cytokine levels	clinical parameters improved after treatment with the probiotic
Probiotics with photodynamic therapy	Mongardini et al. 2017[38]	RCT 6-week follow-up	*L. reuteri* with professionally administered plaque removal and photodynamic therapy	20	20	no significant differences in clinical outcomes between the treatment groups	the adjunctive use of the probiotic did not significantly improve clinical outcomes
Mechanical curettage with photodynamic therapy	Javed et al. 2017[21]	RCT 12-week follow-up	mechanical curettage with/without adjunctive antimicrobial photodynamic therapy	54 test – 28 control – 26	N/A	PI and PPD were significantly higher in the control group ($p < 0.001$)	mechanical debridement with photodynamic therapy is more effective in the treatment of peri-implant mucositis in comparison with mechanical debridement alone
Antimicrobial photodynamic therapy	Al Rifaiy et al. 2018[34]	RCT 12-week follow-up	mechanical debridement and photodynamic therapy (test group) vs. mechanical debridement (control group)	38	65	reductions in PI ($p < 0.001$) and PPD ($p < 0.001$) in the test group as compared to the control group	antimicrobial photodynamic therapy is more effective in comparison with manual debridement alone
Low-abrasive air polishing	Al Ghazal et al. 2017[36]	single-blind RCT	low-abrasive air polishing vs. debridement with titanium curettes	18 test – 9 control – 9	25 test – 15 control – 10	no difference in BoP between the groups ($p = 0.350$)	both treatment methods were proven to be effective in reducing peri-implant inflammation
Air-abrasive debridement	Lupi et al. 2017[40]	RCT 6-month follow-up	maintenance treatment with glycine powder air-abrasive debridement vs. manual debridement and chlorhexidine administration	46	88	air-abrasive debridement significantly improved PI, BoP, PPD, and BS ($p < 0.05$)	treatment with glycine powder seems to be more effective than traditional treatment with plastic curettes and chlorhexidine
Air polishing	Riben-Grundstrom et al. 2015[41]	RCT	glycine powder air polishing vs. ultrasonic debridement	37	37	at 12 months, there were statistically significant reductions in the mean PI, BoP and the number of periodontal pockets ≥4 mm within the treatment groups in comparison with baseline	non-surgical treatment with air polishing or ultrasonic debridement is effective
Enamel matrix derivative	Kashefimehr et al. 2017[37]	double-blind RCT 3-month follow-up	mechanical debridement with enamel matrix derivative vs. mechanical debridement alone	41	41	significant improvement in terms of BoP and PPD in the test group as compared to the control group ($p < 0.0001$)	complete recovery was not observed using either treatment approach
Subgingival ozone and/or hydrogen peroxide	McKenna et al. 2013[45]	double-blind RCT	effect of subgingival ozone and/or hydrogen peroxide on the development of peri-implant mucositis	20	80	significant differences in plaque and modified gingival and bleeding indices were observed between various kinds of treatments	ozone showed great potential for the management of peri-implant mucositis

Treatment	Authors, year of publication	Study type	Study description	Sample size	Implant number	Results	Conclusions
Systemic antibiotics	Hallström et al. 2012[27]	RCT 6-month follow-up	non-surgical treatment of peri-implant mucositis with/without systemic antibiotics	48	N/A	the statistical analysis failed to demonstrate differences in PPD at 6 months	no short-term differences were found between the 2 study groups; the study does not provide evidence for the beneficial effect of systemic antibiotics
Azithromycin	Gershenfeld et al. 2018[33]	RCT 6-month follow-up	mechanical debridement and systemic azithromycin vs. mechanical debridement and placebo	17 test – 9 control – 8	66	the treatment patients showed a consistently greater reduction of gingival inflammation and improvement in soft tissue healing than the control patients	the adjunctive use of azithromycin can assist in the control of peri-implant mucositis
Mechanical debridement	Serino et al. 2018[47]	7-month prospective cohort study	effect of submucosal mechanical instrumentation following supramucosal plaque removal	44	175	at 1 month following supramucosal plaque removal, the number of treated implants with BoP was reduced with a concomitant decrease in the mean PPD value, following submucosal instrumentation, a further reduction in BoP was recorded with a concomitant reduction in the mean PPD value at the 7-month examination	improvement in the clinical condition appeared to be in a large extent due to supramucosal plaque removal
Biofilm control	Gomes et al. 2015[48]	longitudinal cohort study	comparison of the gingival and peri-implant mucosal inflammatory response to mechanical biofilm control	22	N/A	VPI, mPI and gingival bleeding indexes reduced from day 0 onward	supragingival/ supramucosal biofilm control benefited both the teeth and the implants
Photodynamic therapy	Zeza et al. 2018[49]	CCT	professionally administered plaque removal and photodynamic therapy	20	20	a reduction in the median number of BoP sites around implants from3.5 to 2.0 ($p = 0.030$)	peri-implant mucositis can be effectively treated with photodynamic therapy
Mechanical debridement and photodynamic therapy	Al Amri et al. 2016[50]	CCT	mechanical debridement with/without photodynamic therapy in the treatment of peri-implant inflammation in T2DM patients	67 test – 34 control – 33	N/A	BoP and PPD were significantly lower in the test group than in the control group at all follow-ups	in patients with T2DM, mechanical debridement with adjunctive antimicrobial photodynamic therapy is more effective in the treatment of peri-implant inflammation in comparison with mechanical debridement alone
Mechanical debridement paired with diode laser application	Lerario et al. 2016[51]	CCT	conventional treatment with diode laser application (test group) vs. conventional treatment alone (control group)	27	N/A	a reduction of pathological sites from 89% to 14.35% in the test group and from 75.69% to 50% in the control group	diode laser seems to be a valuable tool for peri-implant mucositis treatment
DMT	Chan et al. 2019[52]	case–control study	assessing the modifying effect of DMT on the induction and resolution phases of experimental peri-implant mucositis at DMT ≥ 3 mm (case) and DMT ≤ 1 mm (control)	19	N/A	the removal of the crown and professional submucosal cleaning were necessary to revert to the baseline gingival index in the tested implant	a longer mucosal tunnel results in a much more difficult resolution of peri-implant mucositis

L. reuteri – *Lactobacillus reuteri*; T2DM – type 2 diabetes mellitus; DMT – depth of the implant mucosal tunnel; N/A – data not available; PPD – probing pocket depth; mBI – modified bleeding index; BoP – bleeding on probing; VPI – visible plaque index; mPI – modified plaque index; PI – plaque index; RANKL/OPG – receptor activator of nuclear factor kappa B ligand/osteoprotegerin; *P. gingivalis* – *Porphyromonas gingivalis*; BS – bleeding score.

Discussion

The prevalence data found in this literature review revealed a wide gap in percentage ranges. This could be due to the relevant heterogeneity of the prevalence reported among the 13 cohort studies and 20 cross-sectional studies. Other aspects to consider in order to explain this gap are the sample size and the population observed. Some articles addressed a population composed of smokers or subjects affected by diabetes mellitus; both smoking and diabetes mellitus are well-known periodontal risk factors.

Comparing the results of this review regarding the prevalence of peri-implant mucositis to the prevalence of gingivitis provided by the U.S. National Center for Health Statistics (38.70% PB), a tight overlap can be observed.[9,15]

According to the available data, the average prevalence values for gingivitis and peri-implant mucositis look very similar. This observation is in contrast with the results of a recent study investigating clinical and biological responses in experimental gingivitis and peri-implant mucositis in humans.[16] Although less biofilm accumulation was observed at the implant sites, the peri-implant mucosa yielded a higher proportion of BoP sites as compared to the gingiva.[16] This result probably indicates that less visible plaque accumulation is needed for peri-implant mucositis to develop and that the lack of keratinized gingiva, which is a frequent condition around implants, leading to a weaker seal, can contribute to biofilm migration. This would make the onset and progression of peri-implant mucositis easier and faster than in the case of gingivitis. A possible explanation of this discrepancy is that signs of peri-implant mucositis are generally rarely identified because of the great morphological variability of the overhanging prosthesis.

With regard to prosthodontics, it must be emphasized that it definitely plays a crucial role in mucosal homeostasis. Design, structural connections and constituent materials are all factors concretely correlated to plaque accumulation and the soft tissue response. This heterogeneity may help explain the wide gap in peri-implant mucositis percentage ranges found in this review.

During the present investigation, a general deficiency of the available data on this topic emerged, suggesting more focused research is needed in the future, with a general recommendation for more detailed information in the upcoming studies about peri-implant mucositis.

Another relevant aspect concerns the varying clinical indicators used by different studies. Plaque index (PI), BoP, probing pocket depth (PPD), and marginal recession are not always accompanied by radiological examinations to exclude the presence of peri-implantitis. Therefore, it is advisable to collect all the biometric parameters of signs of inflammation, such as redness, swelling, bleeding, and suppuration, and support them with periodontal indices (BoP and PPD) and radiographic examinations.[17]

These limitations are stressed and partially addressed by the 2017 classification of periodontal and peri-implant diseases and conditions.[18] It is literally cited that "a local dot of bleeding resulting from probing may be the result of a traumatic probing that should not be considered, in the absence of other inflammatory changes, a definitive criterion to characterize a peri-implant soft tissue lesion".[8] For a correct examination, it is consequently crucial to perform circumferential peri-implant probing, using the walking probe method, and to collect all clinical and radiographic parameters to evaluate them as a whole before formulating a diagnosis.

Therefore, considering that attaining a peri-implant mucositis diagnosis seems more complex than a gingivitis diagnosis, the above reported similar prevalence data leads one to presume that the peri-implant mucositis prevalence rates might be underestimated, resulting in a lower clinical perception of this pathology.

Peri-implant mucositis treatment protocols should focus on infection control and the decontamination of the implant surface. Bacterial plaque and calculus must be professionally removed, and the patient must be instructed and motivated to perform proper oral hygiene procedures at home. While gingivitis treatment could achieve restitutio ad integrum through professional hygiene care, mechanical debridement and comprehensive home care, peri-implant mucositis treatment appears more complex, requiring several treatment modalities and devices. Many treatment procedures are performed in association with mechanical debridement, using ultrasonic devices with dedicated polyetheretherketone-coated tips and implant-friendly instruments, such as titanium-coated, carbon-fiber, teflon, and plastic curettes. Also, air-abrasive devices or lasers can be used in conjunction with local antibiotics or antiseptics.[11,19]

In the treatment of gingivitis, scaling and periodontal debridement are able to remove bacterial plaque and calculus from the tooth surfaces, allowing proper healing. None of the proposed therapies for peri-implant mucositis presented in this review led to a complete or strongly predictable resolution, but mechanical debridement accompanied by an adjunctive therapy, such as probiotics, chlorhexidine or photodynamic therapy, proved to provide additional improvement over mechanical debridement alone.[20,21] Galofré et al. compared the effect of the oral probiotic *Lactobacillus reuteri* as an adjuvant to non-surgical mechanical therapy.[20] In their triple-blind RCT, oral probiotics and mechanical therapy together produced additional improvement over treatment with mechanical therapy alone.[20] Also, Javed et al. investigated the outcome of mechanical curettage with or without the adjunct of antimicrobial photodynamic therapy.[21] Forty-four patients were involved in this RCT study, and after 12 weeks of follow-up, mechanical debridement with photodynamic therapy was determined to be more

effective in the treatment of peri-implant mucositis as compared with mechanical debridement alone.[21]

Another promising proposed treatment modality is the use of glycine powder air-polishing devices, which were demonstrated to be as effective as mechanical debridement in a study by Schwarz et al.[22] The same study group, after an electronic and manual search, selected 7 studies which showed that other therapies added to professionally administered plaque removal were quite promising.[23]

A proper prosthetic design that allows good oral hygiene and low plaque accumulation is certainly a key factor in the prevention of peri-implant mucositis. De Tapia et al. reported that when peri-implant tissue inflammation occurs, the prosthetic design should be assessed and modified if necessary to correct the design defects which may be impeding proper hygiene as well as to diminish biomechanical stress factors if involved.[24] A recent RCT compared peri-implant mucositis treatment through chitosan brushes on oscillating handpieces and titanium curettes; a chitosan brush seems to be a safe and efficient device for the debridement of dental implants.[25] Likewise, the regular use of a toothpaste containing triclosan appears to be able to reduce the clinical signs of inflammation in the mucosa adjacent to dental implants.[26] Finally, it has been shown that there is a minimal difference between the non-surgical treatment of peri-implant mucositis with and without systemic antibiotics.[27]

Conclusions

Currently, the available information on the prevalence rates and the standardized therapeutic protocols for peri-implant mucositis are insufficient. Also, it can be presumed that the prevalence rates may be underestimated due to difficulty with making a clinical diagnosis, leading to a lower level of perception among practitioners.

Peri-implant mucositis is a frequently encountered condition. The absence of effective standardized therapeutic procedures that would result in an empirical choice of therapeutic modalities may lead to diminished effectiveness and unsatisfactory treatment outcomes.

It has to be emphasized that implant placement and prosthetic restorations must allow for proper cleaning and plaque control to prevent peri-implant mucositis.

Further research is needed to improve clinicians' skills in the detection of peri-implant mucositis and to determine effective standardized therapies.

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Regenerative endodontic treatment: A systematic review of successful clinical cases

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Abstract

This systematic review was designed to evaluate and compare successful cases of regenerative endodontic treatment (RET) in terms of etiology, diagnosis, treatment protocols, and signs of success. An electronic search was performed in the PubMed and Google Scholar databases. The search was completed by 2 independent reviewers following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines. All in vivo studies in humans that reported at least 1 successful case of RET were included in this review. Successful RET cases were defined as any case that involved the absence of clinical signs/symptoms of periapical inflammation and the presence of continued root maturation, especially apical closure, after the completion of the initial RET. A total of 250 successful cases of RET from 18 studies were selected in this review. A total of 98 (39%) successful cases were detected at least 2.5 years after the initiation of RET. A total of 239 (96%) successful RET cases were presented with the healing/absence of periapical lesions, and no further treatment was required. Furthermore, 45% of the successful RET cases showed root development maturation (stage V). Finally, the clinical outcomes of these RET cases are presented in this systematic review. Prudent case selection and excellent operative protocols are considered to be essential to achieve successful RET outcomes. Future studies are needed to identify a variety of relevant data, including preoperative, intraoperative and postoperative factors, in order to provide a better understanding of successful cases after RET.

Keywords: endodontic regeneration, immature necrotic teeth, outcome assessment, systematic review

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Introduction

Regenerative endodontics has been suggested as the appropriate treatment approach for immature, necrotic-pulp permanent teeth cases. Most clinical regenerative endodontic treatment (RET) cases reported in the literature have presented promising clinical results.[1,2] Radiographic evidence of periapical healing and the lack of clinical signs and symptoms have been recognized as the primary indicators of successful RET.[3] Also, increased root wall thickness and/or length of the immature root, similarly as recovering the vitality of the tooth, have been recognized as additional goals of RET and are associated with a high rate of success.[3] Current endodontic regeneration is frequently referred to as "revascularization". This means cleaning the root canal with the use of an antibiotic mixture and irritating the root apex tissue to create a blood clot inside the root canal that works as a natural scaffold, and to assist pulp–dentin stem cell proliferation and differentiation.[4–6]

Conventional endodontic therapy includes the disinfection, debridement, and subsequent obturation of the root canal system with biologically based materials to replace the function of the organ and the live tissue within the diseased system as an important objective of regeneration in the body.[4,7,8] Regenerative endodontics or "regeneration" (the previous terminology also included "revascularization") consists in performing biologically based procedures to replace the necrotic tissue and create a new tooth structure.[4] Additional benefits of achieving this goal are continued root development and/or apical closure (root end development), and ultimately, avoiding "traditional" root canal therapy. For regeneration, the available cells of the body are invigorated to regrow the missing tissue. Stem cells, tissue scaffolds, growth factors, and other ingredients that can be introduced into the root canal system are beneficial adjuncts for the regeneration of the pulp and further development of the roots.[4,8–11]

After the redefinition of RET in its new concept, evidence-based clinical outcomes have been extracted from case reports/series with favorable outcomes. However, the main apprehension related to this level of evidence is that it may not factually represent the true results of RET, considering that unfavorable outcomes are underreported in most cases. In the last 5 years, numerous prospective and retrospective clinical studies linked to RET have been published in the literature.[1,2,12,13] These types of studies contribute to a higher level of evidence related to successful RET outcomes and present a relatively more accurate depiction of successful RET cases. One review study from 2018 concluded that a successful regenerative procedure was achieved by the regeneration of the pulp with root end closure as well as the healing/absence of periapical pathology, and no further treatment being required.[14] However, if signs or symptoms of disease or necrosis of the new pulp/pulp-like tissue occur, any additional apical closure from the regenerative endodontic procedure can promote a more predictable outcome, should root canal therapy later be required. Thus, even "unsuccessful" regeneration can still be beneficial, as any progress from the initial immature-apex state must be acknowledged as a victory.[2,14]

Previous clinical studies and case reports have revealed positive clinical results of the regenerative therapy of immature necrotic permanent teeth.[5,15–22] Managing RET cases requires good treatment planning, and awareness to determine multiple challenges and to prevent any complications that may occur. The presence of an already compromised tooth with necrotic pulp and an open apex represents a major challenge. Other challenges regard the signs and symptoms of healing periapical lesions which occurred after a successful attempt at RET, such as root maturation and/or the resolution of apical infection. To date, a few available review studies from among the peer-reviewed literature have focused on the success rates of different RET cases without covering most of the different factors influencing RET outcomes associated with successful cases.[23–25] Therefore, the present systematic review aimed to systematically collect, compare and evaluate all successful cases of RET mentioned in the literature. It involved highlighting the etiology for the initiation of RET in these cases, the initial diagnosis of successful cases, the treatment protocols used in these cases, the signs of success, and the time needed to successfully treat immature necrotic teeth.

Material and methods

This study was conducted by 2 independent reviewers following the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) guidelines.[26]

Research question

According to the PRISMA guidelines, the following research question was framed:

"Can successful clinical regenerative endodontic treatment cases be used as strong evidence and a promising future approach in the field of endodontic treatment to treat patients with immature necrotic pulp teeth?"

Information sources

An electronic search for articles published in the English language in the years 2015–2020 was performed using the PubMed and Google Scholar databases.

Literature search strategy

The literature search strategy was carried out in July 2020. The search was performed following the PRISMA guidelines, using 2 electronic databases – PubMed and Google Scholar. The electronic search used the Medical Subject Headings (MeSH) terms, which were combined with the Boolean operators ("AND" and "OR"). The following keywords were used: 'immature teeth' OR 'immature tooth' OR 'immature dentition' AND 'pulp revascularization' OR 'pulpal regeneration' OR 'pulp revitalization' OR 'root canal revascularization' OR 'root maturation' OR 'regenerative endodontic' OR 'regenerative endodontic therapy' OR 'regenerative endodontic treatment' OR 'regenerative endodontic procedure' AND 'blood clot' OR 'platelet-rich fibrin' OR 'platelet-rich plasma' AND 'calcified barrier' OR 'apical closure' OR 'root end formation' OR 'root apex closure'. The detailed description of the search strategy is outlined in Table 1.

This systematic review used the available full-text articles that illustrated the relevant searching of PubMed and Google Scholar. Other databases, such as Scopus, CINAHL (EBSCOhost), Web of Science, and ProQuest Dissertations & Theses (PQDT), were not included in the search strategy, since both the PubMed and Google Scholar databases expand beyond International Scientific Indexing (ISI)-listed journals to include additional scholarly sources, such as non-ISI journals and non-indexed articles that may not be shown in specific databases like Scopus, CINAHL (EBSCOhost) or Web of Science.

The following inclusion criteria were applied to determine which articles would be accepted for the study:
- all in vivo studies in humans in which RET was performed;
- studies published in the English language;
- studies published between 2015 and 2020;
- studies that reported at least 1 successful case of RET.

Due to the lack of consensus in the literature as to defining successful outcomes of RET, the success of RET was defined in the present systematic review as any case of RET that included significant root development maturation as well as the healing/absence of periapical pathology, and no further treatment being required.

Articles that met any of the following criteria were excluded:
- review articles;
- in vitro studies;
- editorial, thesis and personal opinion articles;
- articles that did not report any successful RET cases;
- articles that illustrated clinical relevance about RET by means of percentages and samples taken from non-human sources.

Critical appraisal

Both reviewers independently screened the titles and abstracts of the retrieved articles according to the eligibility criteria as well as the PRISMA guidelines. Any disagreement was resolved through discussion among the 2 reviewers until consensus was reached.

Data extraction

The studies were initially selected by reading their respective titles and abstracts. Subsequently, the full texts of the selected articles were analyzed and organized into standardized Microsoft Office Excel worksheets by both reviewers on an independent basis. The following variables were considered: title; abstract; material and methods; type of publication; and main results.

Data items

Data items from the studies was collected and organized into a table with the following information: author and year, study design, number of successful cases, age and gender of the patients with regard to successful cases, tooth type, etiology, diagnosis, treatment protocol used (i.e., irrigation, intracanal medicaments and the inclusion of any specific scaffold), number of visits needed to finish the treatment, time elapsed between finishing RET and the reported success, signs of success, and root development stage (Cvek's classification).

Table 1. Search strategy used in the present study

Database, search characteristics	Search strategy	Results
PubMed – from inception up to July 31, 2020 – all fields – with no limits	#1 S'immature teeth' OR 'immature tooth' OR 'immature dentition' #2 'pulp revascularization' OR 'pulpal regeneration' OR 'pulp revitalization' OR 'root canal revascularization' OR 'root maturation' OR 'regenerative endodontic' OR 'regenerative endodontic therapy' OR 'regenerative endodontic treatment' OR 'regenerative endodontic procedure' #3 'blood clot' OR 'platelet-rich fibrin' OR 'platelet-rich plasma' #4 'calcified barrier' OR 'apical closure' OR 'root end formation' OR 'root apex closure' #5 #1 AND #2 AND #3 AND #4	#5 = 267
Google Scholar – from inception up to July 31, 2020 – all text – TX – with no limits	'immature teeth' OR 'immature tooth' OR 'immature dentition' AND 'pulp revascularization' OR 'pulpal regeneration' OR 'pulp revitalization' OR 'root canal revascularization' OR 'root maturation' OR 'regenerative endodontic' OR 'regenerative endodontic therapy' OR 'regenerative endodontic treatment' OR 'regenerative endodontic procedure' AND 'blood clot' OR 'platelet-rich fibrin' OR 'platelet-rich plasma' AND 'calcified barrier' OR 'apical closure' OR 'root end formation' OR 'root apex closure'	2,090

Assessment of the risk of bias of the included studies

The assessment of the risk of bias was performed using the criteria suggested in the Cochrane Handbook for Systematic Reviews of Interventions (Version 5.1.0).[27] The Cochrane Collaboration recommends a specific tool for assessing the risk of bias in each included study. The 2 review authors determined the risk of bias of the included studies during the process of data extraction. The risk of bias assessment tool includes 7 specific domains: random sequence generation; allocation concealment; blinding of participants and personnel; blinding of outcome assessment; incomplete outcome data; selective reporting; and other bias.

Each domain was assessed as 'low risk', 'unclear risk' or 'high risk'. The overall risk of bias associated with each study was evaluated as follows:

– low risk of bias: all domains were assessed as 'low risk';
– unclear risk of bias: at least 1 domain was assessed as 'unclear risk';
– high risk of bias: at least 1 domain was assessed as 'high risk'.

Synthesis of the results

As mentioned above, tables were prepared with the relevant data included as data items.

The following types of outcomes were measured:

– primary outcomes: elimination of clinical symptoms (pain, swelling and the sinus tract); reduction in radiographic evidence of the presence of apical pathology;
– secondary outcomes: root development, defined as an increase in root length and root wall thickness.

Statistical analysis

Due to the heterogeneity between the included studies, no meta-analysis could be conducted. Therefore, only parametric data involving the age of the patients for all the included studies is provided as mean and standard deviation ($M \pm SD$). The descriptive evaluation of the findings is also shown.

Results

Study selection

A total of 2,357 studies were initially obtained through the keywords, using the databases. Of those, 1,894 were deleted after reading the title and the abstract, as they displayed either duplicity or an unrelated topic. After applying the inclusion and exclusion criteria, 185 articles were assessed for eligibility. Finally, 18 papers were selected to be included in this review. The flow chart of this systematic review is illustrated in Fig. 1.

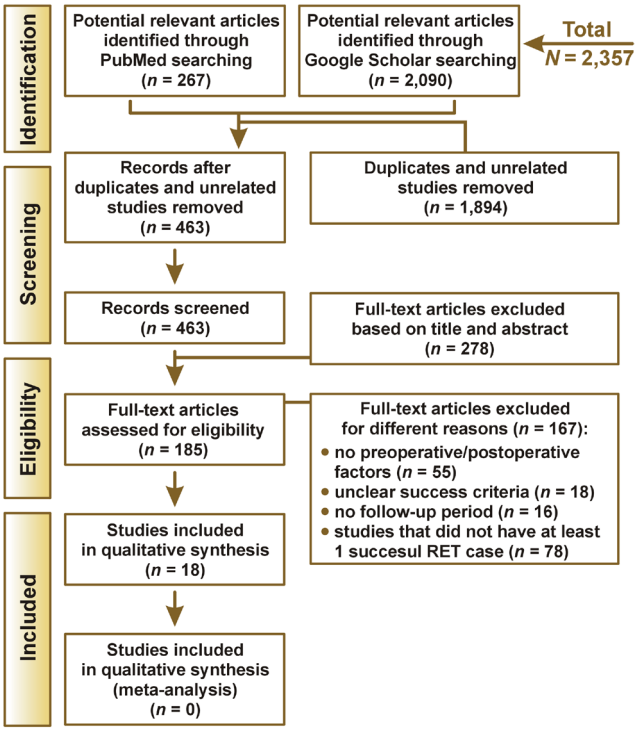

Fig. 1. Flow chart of the search strategy used in this systematic review

Study characteristics

The search ultimately included 18 human studies that satisfied both the inclusion and exclusion criteria and had been conducted within the last 6 years. These studies compared the different factors influencing RET outcomes for successful cases. The 18 included studies had a total sample of 250 successful cases of RET.[13,28–44] The types of studies included in this systematic review were as follows: 7 case reports/case series[28,30,35–37,41,44]; 3 prospective studies[33,39,43]; 5 retrospective studies[31,32,34,38,40]; and 3 randomized clinical trials (RCTs).[13,29,42] The number of successful RET cases ranged from 1 to 45, with the total number of 250. The distribution of these 250 cases was as follows: 10 were extracted from case reports/case series; 74 were identified from prospective studies; 112 were identified from retrospective studies; and 54 cases were identified from RCTs (Table 2). The informative description of all included studies and the different factors influencing RET outcomes for successful cases are summarized in Table 2 and Table 3.

Primary outcomes

Preoperatively, all of the 250 successful cases of RET included across the 18 studies illustrated pretreatment radiographic evidence of periapical pathology.[13,28–44] Postoperatively, clinical signs indicative of infection (pain, swelling and the sinus tract) were absent from 239 (96%) of the RET cases. There was a trend toward a reduction in the size of periapical pathology early after the initiation of RET (reported at less than 1 year) and the complete absence of periapical pathology after at least 2.5 years (Table 3).

Table 2. Summary of the preoperative factors of success and the etiology of regenerative endodontic treatment (RET) cases included in the present systematic review

Authors	Year	Study design	Number of successful cases in the study	Gender (*n*)	Age of patients [years] (for n ≠ 1 *M* ±*SD* or range)	Tooth type	Etiology	Diagnosis
Alasqah et al.[28]	2020	case report	1	male	8	posterior tooth (mandibular first molar)	dental caries	necrotic pulp with asymptomatic apical periodontitis.
Rizk et al.[29]	2019	RCT	26	male (14) female (12)	9.08 ±1.04	anterior teeth (maxillary permanent incisors)	trauma (13) dental caries (13)	necrotic pulp with or without apical pathosis
Ajram et al.[30]	2019	case report	1	female	7	posterior tooth (mandibular first molar)	dental caries	necrotic pulp with symptomatic apical periodontitis
Botero et al.[13]	2017	RCT	9	male (5) female (4)	9.50 ±2.73	anterior teeth (8) posterior toot (1)	trauma (8) dental caries (1)	necrotic pulp
Linsuwanont et al.[31]	2017	retrospective study	13	NR	12.00 ±1.41	anterior teeth (4) posterior teeth (9)	trauma (5) dental caries (2) dens evaginatus (6)	necrotic pulp with an acute apical abscess (1) necrotic pulp with a chronic apical abscess (5) necrotic pulp with asymptomatic apical periodontitis (6) necrotic pulp with symptomatic apical periodontitis (1)
Peng et al.[32]	2017	retrospective study	45	NR	10.8 ±1.92	NA	NR	necrotic pulp with apical pathosis
Chan et al.[33]	2017	prospective study	26	NR	9.20 ±2.41	NA	NR	necrotic pulp with or without apical pathosis
Silujjai and Linsuwanont[34]	2017	retrospective study	13	NR	16.10 ±10.32	(4) anterior teeth (4) posterior teeth (9 – 7 premolars and 2 molars)	trauma (4) dental caries (2) dens evaginatus (7)	necrotic pulp with an acute apical abscess (1) necrotic pulp with a chronic apical abscess (6) necrotic pulp with asymptomatic apical periodontitis (5) necrotic pulp with symptomatic apical periodontitis (1)
Moodley et al.[35]	2017	case report	1	male	10	anterior tooth (maxillary central incisor)	dental fracture	necrotic pulp with an acute apical abscess
Timmerman and Parashos[36]	2017	case report	1	male	16	anterior tooth (maxillary lateral incisor)	dens invaginatus	previously initiated therapy with asymptomatic apical periodontitis
Topçuoğlu and Topçuoğlu[37]	2016	case report	3	male (1) female (2)	8.30 ±0.57	posterior teeth (mandibular first molars)	NR	necrotic pulp with apical pathosis
Chen and Chen[38]	2016	retrospective study	16	NR	10.90 ±0.98	posterior teeth (premolars)	dens evaginatus	necrotic pulp with apical pathosis

Authors	Year	Study design	Number of successful cases in the study	Gender (*n*)	Age of patients [years] (for n ≠ 1 *M* ±*SD* or range)	Tooth type	Etiology	Diagnosis
Estefan et al.[39]	2016	prospective study	33	male (19) female (14)	13.20 ±3.06	anterior teeth (maxillary incisors)	NR	necrotic pulp with or without apical pathosis
Bukhari et al.[40]	2016	retrospective study	25	NR	8–31	anterior teeth (19) posterior teeth (6 – 5 premolars and 1 molar)	trauma (18) dental caries (4) dens invaginatus (3)	necrotic pulp with or without apical pathosis
Nosrat et al.[41]	2015	case report	2	male (1) female (1)	9.50 ±0.70	posterior teeth (maxillary first premolars)	NR	necrotic pulp without apical pathosis
Bezgin et al.[42]	2015	RCT	19	male (10) female (9)	10.1 ±1.85	anterior teeth (13 maxillary incisor teeth) posterior teeth (6 – 1 maxillary second premolar and 5 mandibular premolars)	trauma (13) dental caries (6)	necrotic pulp with or without apical pathosis
Narang et al.[43]	2015	prospective study	15	NR	below 20	NA	NR	necrotic pulp with or without apical pathosis
McCabe[44]	2015	case report	1	female	7	anterior tooth (maxillary central incisor)	trauma	necrotic pulp with symptomatic apical periodontitis

RCT – randomized clinical trial; NR – not reported; NA – not applicable.

Table 3. Summary of the intraoperative and postoperative factors of success of regenerative endodontic treatment (RET) cases included in the present systematic review

Study	Signs of success	Root development stage (Cvek's classification)	Irrigation type	Type of medicament	Number of visits	Capping material used	Scaffold used	Time between the initiation of RET and success
Alasqah et al.[28]	periapical healing and complete root formation	stage V	1.5% NaOCl and 17% EDTA	antibiotic and $Ca(OH)_2$	multiple	MTA	blood clot	2 years
Rizk et al.[29]	completed development of the root apex	stage V	2% NaOCl	antibiotic	multiple	MTA	blood clot (13) PRP (13)	1 year
Ajram et al.[30]	complete periapical healing and apical closure	stage V	10 mL sterile saline and 20% EDTA	$Ca(OH)_2$	multiple	MTA	blood clot	2 years
Botero et al.[13]	apical closure	stage V	2.5% NaOCl and 17% EDTA	$Ca(OH)_2$	single	MTA	blood clot	1 year

Study	Signs of success	Root development stage (Cvek's classification)	Irrigation type	Type of medicament	Number of visits	Capping material used	Scaffold used	Time between the initiation of RET and success
Linsuwanont et al.[31]	continued root development with apical closure in most cases	stage IV (6) stage V (7)	NaOCl and EDTA	antibiotic or $Ca(OH)_2$	multiple	MTA	blood clot	1–8 years
Peng et al.[32]	resolution of periapical lesions and various levels of root development with apical closure or near closure	NA	5.25% NaOCl	antibiotic	multiple	GIC (24) MTA (21)	blood clot	2.5–3 years
Chan et al.[33]	resolution of periapical lesions and various levels of root development with apical closure or near closure in most cases	stage III (4) stage IV (14) stage V (8)	5.25% NaOCl	antibiotic	multiple	MTA	blood clot	2.5 years
Silujjai and Linsuwanont[34]	resolution of periapical lesions and various levels of root development	stage III (4) stage IV (4) stage V (5)	1.5–2.5% NaOCl and 17% EDTA	antibiotic or $Ca(OH)_2$	multiple	MTA	blood clot	3.5 years
Moodley et al.[35]	resolution of apical radiolucency with complete apical closure	stage V	1.5% NaOCl and 17% EDTA	antibiotic and $Ca(OH)_2$	multiple	MTA	blood clot	2–5 months
Timmerman and Parashos[36]	complete periapical healing and complete apex formation	stage V	1% NaOCl and 15% EDTA	$Ca(OH)_2$	multiple	MTA	blood clot	3 years
Topçuoğlu and Topçuoğlu[37]	absence of periapical lesions and evidence of the apical closure of all teeth	stage V	2.5% NaOCl and 17% EDTA	$Ca(OH)_2$	single	MTA	PRP	1.5 years
Chen and Chen[38]	periapical healing and apex formation in most cases	NA	2.5% NaOCl	$Ca(OH)_2$	multiple	MTA	blood clot	1 year
Estefan et al.[39]	periapical healing and an increase in root apical narrowing	NA	2.5% NaOCl and 17% EDTA	antibiotic	multiple	MTA	blood clot	1 year
Bukhari et al.[40]	absence of clinical signs and symptoms; either a reduction in size of periapical lesions, or complete healing and apical closure in most cases	stage IV (4) stage V (21)	3% NaOCl and 17% EDTA	antibiotic	multiple	bioceramic putty or MTA	blood clot	2 years (12) 4–6 years (13)
Nosrat et al.[41]	root development evident in both teeth	NA	1.25% NaOCl and 17% EDTA	antibiotic	single	MTA	blood clot	4 months
Bezgin et al.[42]	apical closure in most cases and evidence of bone healing in all cases	stage IV (6) stage V (13)	2.5% NaOCl, 17% EDTA and 0.12% CHX	antibiotic	multiple	MTA	blood clot (9) PRP (10)	1.5 years
Narang et al.[43]	periapical healing and apical closure in all cases	stage V	2.5% NaOCl	antibiotic	NR	resin-modified GIC	blood clot (5) PRP (5) PRF (5)	1.5 years
McCabe[44]	periapical healing and apical closure	stage V	5% NaOCl and 17% EDTA	NR	single	MTA	blood clot	1.5 years

NaOCl – sodium hypochlorite; EDTA – ethylenediaminetetraacetic acid; CHX – chlorhexidine; $Ca(OH)_2$ – calcium hydroxide; MTA – mineral trioxide aggregate; GIC – glass-ionomer cement; PRP – platelet-rich plasma; PRF – platelet-rich fibrin.

Secondary outcomes

The reported secondary outcomes were variable and included an increase in root length and root wall thickness in 154 (62%) cases. In addition, there was apical closure and complete root development in 112 (45%) cases (Table 3).

Etiology and diagnosis of successful RET cases

In 97 successful cases, the gender of patients was reported (53 cases in males and 44 cases in females). Gender was not stated in the other 153 cases (Table 2). A total of 110 successful RET cases occurred in anterior teeth and 54 in posterior teeth. The tooth location was not indicated in the other 86 cases (Table 2). The etiology for the initiation of RET was not reported in 124 successful cases. On the other hand, a total of 126 successful RET cases reported the etiology resulting in the initiation of RET. Sixty-two (49%) of these cases were caused by dental trauma, 30 (24%) by dental caries, 29 (23%) by dens evaginatus, 4 (3%) by dens invaginatus, and 1 (0.8%) by dental fracture (Table 2). All successful cases reported a pulpal diagnosis with necrotic pulp, except for 1 case,[36] which reported previously initiated therapy. Most of the successful cases that reported a periapical diagnosis had some type of apical pathosis. Only 2 cases reported normal apical tissue, 3 cases were diagnosed with an acute apical abscess, 11 cases reported a diagnosis of a chronic apical abscess, 13 cases were diagnosed with asymptomatic apical periodontitis, and 4 cases were diagnosed with symptomatic apical periodontitis. Also, 64 cases were reported "with apical pathosis" and 144 cases were reported "with or without apical pathosis" in the studies, without specifically identifying the type of apical pathology. Only 9 cases did not report an apical diagnosis (Table 2).

Irrigation protocol used in successful RET cases

The use of sodium hypochlorite (NaOCl) as the main irrigation solution with a concentration ranging from 1% to 5.25% was reported in 249 (99.6%) successful RET cases, while 1 (0.4%) case[30] used 10 mL sterile saline and 20% ethylenediaminetetraacetic acid (EDTA). Nineteen (8%) cases used chlorhexidine (CHX) irrigation with a concentration of 0.12%. A total of 128 (51%) successful cases reported that NaOCl was the only irrigation solution used in the procedure, 102 (41%) of the successful cases used various combinations of NaOCl and EDTA, and 19 (8%) of the successful cases used various combinations of NaOCl, EDTA and CHX. A total of 128 (51%) successful RET cases did not use EDTA in their irrigation protocols (Table 3). The type of intracanal medicament was reported in 249 (99.6%) successful cases, and only 1 (0.4%) case[43] did not report the type of intracanal medicament used in the procedure. Calcium hydroxide ($Ca(OH)_2$) was the only intracanal medicament used in 30 (12%) cases, and 2 cases (0.8%) used a combination of an antibiotic and $Ca(OH)_2$. Twenty-six (10%) cases used an antibiotic combination or $Ca(OH)_2$ in their studies, and 191 (76%) successful cases reported that an antibiotic was the only intracanal medicament used in the regenerative endodontic procedure (Table 3). In 15 (6%) cases, RET was finished in a single visit, whereas in 220 (88%) cases, the completion of RET required multiple visits. Only for 15 (6%) successful cases, the number of visits was not reported (Table 3).

Scaffold and capping materials used in successful RET cases

A total of 214 (86%) successful cases used a blood clot as a scaffold. Thirty-one (12%) successful cases reported the use of platelet-rich plasma (PRP) in the RET protocol and 5 (2%) successful cases reported the use of platelet-rich fibrin (PRF) (Table 3). A total of 186 (74%) successful cases reported that mineral trioxide aggregate (MTA) was the only capping material used in the procedure. Twenty-four (10%) cases used glass-ionomer cement (GIC), 25 (10%) cases used various combinations of capping materials with bioceramic putty or MTA and 15 (6%) cases used resin-modified GIC in their RET (Table 3).

Signs of success and root development stage of successful RET cases

The reported time elapsed between the initiation of RET and the identification of successful cases ranged from 2 months to 8 years (Table 3). Three (1%) successful cases were detected less than 1 year after the initiation of RET, 84 (34%) successful cases were detected immediately after 1 year since the initiation of RET, 38 (15%) successful cases were detected 1.5 years after the initiation of RET, 14 (6%) successful cases were detected 2 years after the initiation of RET, and 98 (39%) successful cases were detected at least 2.5 years after the initiation of RET. Only 13 (5%) successful cases ranged the time of success from 1 year to 8 years, without specifically identifying the time for each case (Table 3). A total of 239 (96%) successful cases reported the healing/absence of different periapical lesions as the main sign of success (Table 3). Due to the instructive radiographic features of Cvek's classification and the fact that it illustrates clinical outcomes better than other classification schemes,[45] Cvek's classification was used in this review to determine the root maturation stage for all of the successful RET cases. The distribution of root development stages according to Cvek's classification was as follows: stage V (completed apical closure) was reported as a sign of success in 112 (45%) cases; stage IV was reported in 34 (14%) cases; stage III was reported in 8 (3%) cases; and for 96 (38%) cases, the root development stage was not reported (Table 3).

Assessment of the risk of bias of the included studies

The Cochrane Collaboration's tool for determining the risk of bias was used for all the studies included in this review.[27] The 2 authors integrated the information and summarized the risk of bias associated with the included studies. The majority of studies had a low risk of bias concerning the following domains: blinding of outcome assessment (94%); incomplete outcome data (66%); selective reporting (77%); and other bias (88%). All of the studies presented a low risk of bias (100%) with regard to the random sequence generation, allocation concealment, and blinding of participants and personnel domains (Fig. 2). In summary, among the 18 studies assessed for the overall risk of bias, not one study was classified as having a high risk of bias; 11 (61%) studies were considered to have a low risk of bias and 7 (39%) studies had an unclear risk of bias (Fig. 3). The scoring of unclear risk of bias was given to 7 studies, as there was not enough information to make a clear judgment concerning the following domains: blinding of outcome assessment; incomplete outcome data; selective reporting; and other bias (Fig. 3). A review of the authors' judgments about each risk of bias domain is presented in Fig. 2. Finally, the risk of bias for each study is presented in Fig. 3.

Discussion

This systematic review was carried out to summarize and appraise all studies published within the last 6 years that were relevant to our study aim. It gathered all recent clinical studies that investigated the factors influencing RET outcomes for successful cases. This study demonstrates a comprehensive set of evidence extracted from 18 articles that fulfilled our inclusion and exclusion criteria. To date, 2 systematic reviews have discussed the signs of success in different human and animal studies concerning the preoperative factors and postoperative factors in RET (Table 4).[24,25] Alghamdi and Alqurashi concluded in their systematic review that most of the retrieved studies about RET suggested its effectiveness in periapical healing and the formation of apical closure in immature necrotic teeth.[25] In addition, Torabinejad et al. demonstrated in their systematic review that the primary goal of RET could be reliably achieved with high probability (91–94% of periapical healing).[24] This clearly shows consistency in the conclusions drawn by the previously published systematic reviews. In agreement with those findings, 239 (96%) cases in this review showed the healing/absence of different periapical lesions after RET in human subjects. Also, the 2 abovementioned articles covered the period between 1966 and 2019 (Table 4), while our systematic review covered all eligible articles published within the last 6 years (Table 2 and Table 3).

In our systematic review, most of the successful cases mentioned the etiology for the initiation of RET (Table 2). Dental trauma accounted for 49% of these cases as the main etiology for the initiation of RET, followed by the existence of dental caries (24%) and dens evaginatus (23%). These outcomes are in agreement with a previously published review, which found that the etiology of 30% of all cases treated with RET was dental trauma, followed by the presence of dens evaginatus (22%).[46] However, for the majority of successful RET cases, the type of traumatic injury was not reported.[46] One clinical study concluded that there were significantly better outcomes for RET cases with an etiology of dens evaginatus as compared to dental trauma.[2] The current systematic review found that the healing/absence of periapical lesions was the main presentation of RET success in 96% of cases, as one of the signs of RET success (Table 3). In addition to other signs of success for these RET cases (Table 3), apical closure formation is the secondary goal of RET and the second sign of success in 45% of successful RET cases (Table 3) in comparison with other traditional treatment options for the management of immature necrotic teeth, including periapical surgery and apexification.

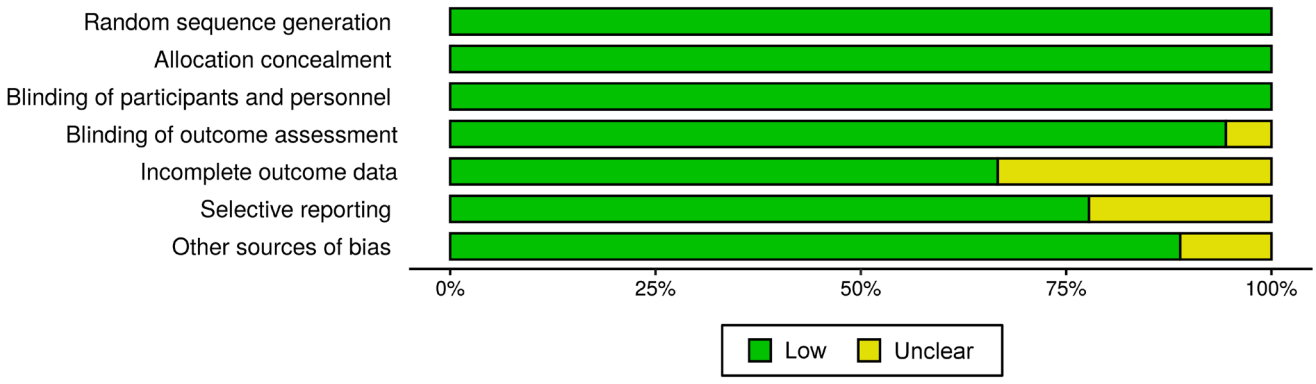

Fig. 2. Risk of bias graph

The review authors' judgments about each risk of bias item are presented as percentages across all the included studies. Green and yellow refer to a low risk of bias and an unclear risk of bias, respectively.

Fig. 3. Risk of bias summary

The review authors' judgments about each risk of bias item for each included study. Green and yellow refer to a low risk of bias and an unclear risk of bias, respectively.

Other publications point out that maintaining a high level of disinfection intra/postoperatively in RET is important to enhance RET outcomes.[2,47–50] Antibiotic medicaments and high-concentration irrigation solutions are recommended to improve the irrigation protocols during RET, since NaOCl,[51] CHX,[52] and antibiotic intracanal medicaments[53] have concentration-dependent antibacterial effects that contribute to reducing different

Table 4. Summary of the 2 systematic reviews included in the present review

Authors	Year	Number of studies included	Method summary	Main conclusions
Alghamdi and Alqurashi[25]	2020	46 studies	The systematic review presents and summarizes human and animal studies performed from 2009 to 2019 retrieved by means of the electronic search of 2 databases (PubMed and Google Scholar).	RET was more efficient in treating immature necrotic $permanent teeth and offered a greater advantage, which should lead to wider acceptance among endodontists with regard to effective results in comparison with other treatment options.
Torabinejad et al.[24]	2017	144 studies	The systematic review presents and summarizes in vivo human clinical studies performed from 1966 to November 2016 retrieved by means of the electronic search of 3 databases (PubMed, Web of Science and Cochrane Library).	The treatment of immature teeth with pulp necrosis with the use of MTA or RET shows high survival and success rates.

biofilms produced by endodontic pathogens. However, the main irrigation predicament in RET is that lower concentrations of various intracanal medicaments and irrigation solutions are advised to preserve the survival of stem cells from apical papillae.[54–57] Thus, different irrigation approaches should be examined in an endeavor to reach lower concentrations of antibacterial irrigations and medicaments that can still maintain a high level of irrigation.

In our systematic review, most of the successful RET cases had a follow-up period ranging from 2 months to 8 years. In contrast, there is a previous review that retrieved 18 successful cases with RET and reported the history of pulp necrosis no longer than half a year.[58] Also, in the present study, the time elapsed between the initiation of RET and the recognition of successful RET was exactly 1 year in 34% of successful RET cases. Furthermore, 39% of all the included successful RET cases were identified at least 2.5 years after the initiation of RET. Some of the successful RET cases reported evidence of initial favorable outcomes, such as the resolution of radiographic lesions,[34,35,41] apical closure,[35,41] and an increase in root length and root thickness.[34,35,41] Several case reports and retrospective clinical studies in the literature have reported a high success rate for RET after a maximum follow-up period of 12–19 months.[1,2,12,42,59] In one of the systematic reviews, they calculated the average follow-up time of RET studies to be 16.7 months.[24]

In our systematic review, 15 successful RET cases were completed in a single visit with different intracanal medicaments used.[13,37,41,44] These results are in agreement with a previously published case report, in which RET was performed in a single visit and was reported to be successful.[60] A total of 74% of successful RET cases reported the use of MTA as the main capping material in their studies. Other studies used different capping materials, including GIC (10% of cases) and resin-modified GIC (6% of cases). A comparative systematic review found that the pooled success rates for MTA apical plugs and RET were 94.6% and 91.3%, respectively.[24]

In summary, this systematic review was developed based on the comparison of the impact of the preoperative and postoperative factors on eventual treatment success in RET (Table 2 and Table 3). Some studies used radiographic evidence of completed apical closure and the healing of periapical lesions to define the successful outcomes of RET cases. There is heterogeneity in the RET protocols used to manage the cases reported in this review. Thus, following the updated evidence-based clinical considerations from the American Association of Endodontists (AAE), it is compulsory to reduce variability in RET protocols.[57] Finally, it can be concluded that RET can be a useful tool in the field of endodontics along with other approaches in treating children and young adult patients with immature necrotic teeth. The successful clinical RET cases discussed in this review can be considered as high-level evidence in the field of endodontics with regard to patients with immature necrotic-pulp teeth. Based on the present evidence, it seems that the treatment of immature teeth with pulp necrosis by means of RET provides high survival and success rates.

Study strengths and limitations

This study summarized and appraised all peer-reviewed studies published within the last 6 years that fulfilled both our inclusion and exclusion criteria. To our knowledge, this is the only systematic review that covered the topic of the different factors influencing RET outcomes for successful cases in depth. The systematic reviews conducted by Alghamdi and Alqurashi[25] and Torabinejad et al.[24] covered the periods of 2009–2019 and 1966–2016, respectively (Table 4). Our review used PubMed and Google Scholar as search engines. One advantage of using Google Scholar is that it ensures that reviewers do not miss any relevant research published in journals that are still not cited in PubMed. Unfortunately, due to the heterogeneity of the influencing factors in the existing human studies, we were restricted to performing a systematic review without a meta-analysis. Finally, although studies conducted on human subjects were part of the inclusion criteria, we unfortunately could find but a few published successful case studies that used RET to treat children and young adult patients with immature necrotic teeth.

Conclusions

Several RET cases of immature necrotic teeth were reported in this review. These cases provide clinical outcomes, such as apical closure formation, the healing of different forms of apical pathology as well as a significant follow-up duration. Thus, prudent case selection and excellent operative protocols are considered to be essential to achieve the successful outcomes of the RET procedure. Collectively, this systematic review showed that the healing/absence of periapical pathology was the main measure of success in 96% of successful RET cases. Furthermore, 45% of successful RET cases presented with root development maturation Stage V, and 39% of successful RET cases were identified at least 2.5 years after the initiation of RET. Future studies should describe a variety of relevant data, including preoperative, intraoperative and postoperative factors, to provide a better understanding of successful cases after RET.

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