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Original paper

Sexuality and intimacy after head and neck cancer treatment: An explorative prospective pilot study

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Abstract

Background. While sexuality and intimacy are suggested to contribute to quality of life (QoL), it is striking that the sexual problems of head and neck cancer patients have not been adequately studied.

Objectives. Our aim was to prospectively assess the impact of head and neck cancer and its treatment on sexuality and intimacy.

Material and methods. A questionnaire study with a 6-month follow-up period was conducted at the University Hospitals Leuven, Belgium, using the Maudsley marital questionnaire (MMQ), the sexual adjustment questionnaire (SAQ) and the short sexual functioning scale (SSFS) to prospectively assess the impact of head and neck cancer and its treatment on sexuality and intimacy.

Results. Twelve patients (67%) reported a negative impact on their sexuality and/or intimacy. There were significant declines in marital, sexual and general life satisfaction ($p < 0.000$) at the 6-month follow-up as compared to baseline. There was a significant increase in frustration after sexual activity ($p = 0.031$). Sexual desire was also impacted, with a near doubling of patients reporting a decline. The perceived importance of discussing sexual issues with one's physician significantly increased from 7 to 16 patients ($p = 0.004$).

Conclusions. Sexual problems are common after head and neck cancer treatment. Using a screening instrument can help to identify patients that need intervention. Discussing sexuality and intimacy issues that patients may face before, during and after treatment can have a positive impact on QoL.

Keywords: sexual dysfunction, sexuality, intimacy, head and neck cancer

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Introduction

Head and neck cancer accounts for approx. 2% of all cancers in Europe.[1] As head and neck cancer is characterized by slow progression and insidious symptoms, patients are often diagnosed at a late stage (III or IV).[2,3] This leads to greater disease severity at diagnosis, and increased treatment complexity and morbidity. Patients with head and neck cancer have a poor prognosis, with current 5-year survival rates ranging from 35% for oral tumors to 60% for larynx tumors.[4] The aforementioned late diagnosis combined with the currently available invasive treatment options, i.e., surgery and radiotherapy with or without chemotherapy, result in the disease itself and its treatment having a significant impact on patients' quality of life (QoL). To ensure optimal QoL, an early diagnosis and strict follow-up consultations are essential. An early diagnosis is often facilitated by a well-trained dentist or general practitioner. Non-invasive, radiation-free imaging, such as ultrasound, can be a valuable tool in primary care to identify malignancies at an early stage.[5] Patients with suspect lesions can be promptly referred to a maxillofacial or head and neck surgeon. Additionally, magnetic resonance imaging (MRI) can be conducted. It is the gold standard, radiation-free imaging technique for soft tissue tumors.[6]

In the follow-up of head and neck cancer patients, several questionnaires, including the European Organization for Research and Treatment of Cancer quality of life questionnaire – head and neck (EORTC QLQ-H&N35) and the University of Washington quality of life questionnaire (UW-QOL), are often used to measure the QoL of patients. As these questionnaires focus mainly on pain, dysphagia, mucositis, xerostomia, appearance, and speech and chewing capabilities, other important determinants of QoL, such as sexuality and intimacy, are mostly not addressed.[7] This is striking, as the head and neck region is important with regard to appearance, communication with one's partner and kissing, which are vital aspects in the domain of sexuality and intimacy.

The impact of a cancer diagnosis is often felt also by partners, families and in a broader social context.[8] Many patients experience feelings of anger, anxiety, guilt, depression, hopelessness, and fear of having to depend on others.[9] Complex treatment, which is necessary, can lead to disfigurement and the loss of function.[10] The direct and indirect physiological and psychological effects of cancer may alter sexual functioning and sexual experience. The partner's preoccupation with managing a cancer patient's care and frequent hospital visits can result in less sexual activity in the relationship.[11] Pain and fatigue, before and/or after treatment, can further exacerbate sexual disinterest. Cancer itself may lead to sexual dysfunction, but most problems are attributed to the treatment used, i.e., surgery, radiotherapy and chemotherapy.[12]

Most of the literature pertaining to sexuality and intimacy in cancer patients focuses on gynecological and urological malignancies. Half of these patients report severe sexual dysfunction, including decreased desire, arousal and lubrication, orgasmic concerns, dyspareunia, and erectile dysfunction.[13] It is worth noting that sexuality and intimacy are a concern for most patients, regardless of age.[14] Due to the prevalence of sexual dysfunction in prostate and gynecological cancer patients, the need to provide information and counseling about such issues has already been recognized for these patient groups. A specific expanded prostate cancer index composite (EPIC) questionnaire has been created to diagnose patients with sexual dysfunction, to assess the degree of sexual dysfunction and to create a treatment plan.[15]

In head and neck cancer care, the information and counseling regarding sexuality and intimacy given before, during or after treatment remain limited. Moreover, studies regarding the sexuality of head and neck cancer patients are scarce. A retrospective study specifically evaluating sexual dysfunction in head and neck cancer patients, using a modified version of the sexual adjustment questionnaire (SAQ) was conducted in Cincinnati, USA, from 2008 to 2009.[16] In that study, 42 patients were interviewed 4 months after treatment; all of them reported a negative impact on their sexual relationships due to head and neck cancer, of whom 50% reported an extremely negative impact. Those who underwent surgical treatment were more sexually satisfied as compared to those who were treated with radiotherapy and/or chemotherapy.[16] Researchers in Norway and Sweden examined the QoL of 357 head and neck cancer patients using EORTC QLQ-H&N35 at baseline and at 1, 2, 3, 6, and 12 months after treatment (surgery, radiotherapy, chemotherapy).[17] The aim of that study was to assess what problems the patients faced and for how long. Among other things, the patients reported a significant reduction in sexual function. Unlike in the case of many other problems, e.g., pain, the loss of appetite and fatigue, some of the sexual problems reported by the patients persisted from baseline to the 12-month assessment time point.[17]

Currently, most patients with head and neck cancer are middle-aged men. In 1983, a causal relationship was identified between the human papillomavirus (HPV), which is usually contracted during puberty, and oropharyngeal squamous cell carcinoma (SCC).[18] It has been related to a shift in the age of head and neck cancer patients. Younger patients have to cope with long-term treatment side effects for an extended period of time. Head and neck cancer treatment induces specific physiological, psychological, communicational, and relational changes that can affect a patient's ability to be intimate with their partner. Surgery can lead to disfigurement, scarring, changed facial expressions,

altered innervation, and diminished speaking, chewing and swallowing capabilities. Due to advances in technology, the side effects of radiotherapy have been significantly reduced by the use of intensity-modulated radiotherapy (IMRT). However, mucositis, xerostomia and osteonecrosis of the jaw remain considerable side effects. Chemotherapy can cause acute side effects, such as nausea, vomiting and fatigue, which can affect patients' sexual desire and sexual functioning.[4,19]

Therefore, the aim of the current study was to estimate the prevalence of problems related to sexuality and intimacy after head and neck cancer treatment. We expect that a majority of head and neck cancer patients experience intimacy problems due to treatment-related side effects. Moreover, this study aimed to determine which patients would benefit from additional information and could potentially be helped by referral to a sexologist. Very few prospective studies focusing on sexuality and intimacy within this specific patient population have been conducted.

Material and methods

Study design

The current research consisted of a survey study, including the questionnaires assessing patient sexual functioning (an adapted version of the short sexual functioning scale (SSFS) and an adapted version of SAQ), and marital and sexual satisfaction (the Maudsley marital questionnaire (MMQ)). The inclusion criteria were as follows: adults aged 18 years and above; and a primary head and neck cancer diagnosis (i.e., oral cavity, pharynx, larynx, paranasal sinuses, salivary glands, thyroid gland, or unknown primary) from September 2016 to January 2017 at the University Hospitals Leuven, Belgium. The cancers included T1–4, N0–3, M0 disease, mostly histologically confirmed SCC, but other histological types were also eligible. The patients were recruited from the departments of maxillofacial surgery, otorhinolaryngology and radiotherapy-oncology. The patients needed to be available to attend a long-term follow-up. The following exclusion criteria were applied: patients with recurrent head and neck cancer that was already treated with surgery, radiotherapy and/or chemotherapy; patients diagnosed with depression according to the "Diagnostic and Statistical Manual of Mental Disorders: DSM-5™" (5th ed.) criteria[20]; patients with cognitive impairment that resulted in an inability to complete the questionnaires; and patients with previous or concurrent illnesses that could interfere with follow-up (Table 1). The respondents were interviewed the day before their initial treatment (surgery, radiotherapy and/or chemotherapy). Pre-treatment sexual assessment screening is essential because of varying baseline (pre-existing) sexual functioning and satisfaction among patients. Six months after the completion of treatment, the respondents were asked to complete the questionnaires again to evaluate changes as compared to the baseline scores. The study protocol and procedure were approved by the Ethics Committee at the University Hospitals Leuven (No. of approval: S-59565).

Patient characteristics

The patient characteristics are presented in Table 2. In total, 45 head and neck cancer patients were reviewed for inclusion, of whom 26 had a new diagnosis. Of these 26 patients, 7 declined (1 man and 6 women). In total, 19 men completed the questionnaires before treatment, of whom 18 (mean age: 62.1 years) also completed the questionnaires at the follow-up 6 months post-treatment. One patient died within the 6-month follow-up period. At baseline, 15 patients (83%) reported having a partner; one patient's partner passed away between the baseline and follow-up questioning. While almost all participants (n = 16; 89%) reported a history of smoking (mean pack years: 31.3), 6 patients (33%) were still active smokers during the study period. Fourteen patients (78%) reported daily alcohol consumption and 6 patients (33%) had a history of alcohol abuse.

Table 1. Patient inclusion and exclusion criteria

Criteria	Description
Inclusion criteria	– age 18 years and above – primary head and neck cancer diagnosis (i.e., oral cavity, pharynx, larynx, paranasal sinuses, salivary glands, thyroid gland, or unknown primary) – T1–4, N0–3, M0 disease, mostly histologically confirmed SCC, but other histological types were also eligible – available to attend a long-term follow-up
Exclusion criteria	– recurrent head and neck cancer that was already treated with surgery, radiotherapy and/or chemotherapy – depression according to the "Diagnostic and Statistical Manual of Mental Disorders: DSM-5" (5th ed.) criteria – cognitive impairment with an inability to complete the questionnaires – previous or concurrent illness that could interfere with follow-up

SCC – squamous cell carcinoma.

Table 2. Patient characteristics

Patient	Age [years]	Gender	Cancer site	Stage*	Partner	Current smoker	History of smoking
1	56	male	unknown primary	IVA	no	yes	yes
2	59	male	larynx/hypopharynx	IVA	yes	no	yes
3	56	male	oropharynx	IVA	yes	no	no
4	82	male	larynx/hypopharynx	II	yes	yes	yes
5	73	male	larynx/hypopharynx	IVA	yes	no	yes
6	54	male	oropharynx	IVB	no	yes	yes
7	71	male	oral cavity	IVA	yes	no	yes
8	68	male	salivary gland	II	yes	yes	yes
9	58	male	oral cavity	IVA	yes	no	yes
10	61	male	salivary gland	IVA	yes	no	yes
11	61	male	oral cavity	I	yes	yes	yes
12	65	male	larynx/hypopharynx	IVA	yes	no	yes
13	57	male	thyroid gland	III	no	no	yes
14	46	male	thyroid gland	III	no	no	yes
15	54	male	thyroid gland	IVA	yes	no	yes
16	61	male	oral cavity	IVA	yes	yes	yes
17	64	male	oral cavity	III	yes	no	yes
18	73	male	salivary gland	IVA	yes	no	no

* The head and neck cancer stage defined according to the American Joint Committee on Cancer (AJCC) tumor, node, metastasis (TNM) classification (Amin MB, Edge S, Greene F, et al., eds. *AJCC Cancer Staging Manual*. 8th ed. New York, NY: Springer International Publishing: American Joint Committee on Cancer; 2017).

Cancer type and treatment

The study population had a variety of head and neck cancer sites, with stages ranging from I to IVB. After a multidisciplinary discussion, each patient received a tailored treatment consisting of surgery, radiotherapy, chemotherapy, or a combination of these.

One patient was diagnosed with stage I oral cavity cancer and underwent a tumorectomy. Of the 2 patients with stage II cancer, one was treated with radiotherapy and the other was treated with surgery (parotidectomy and neck dissection) followed by adjuvant radiotherapy. The 3 patients with stage III cancer were treated surgically, including neck dissections; the 2 patients with thyroid carcinoma also received adjuvant iodotherapy, whereas the third patient (SCC of the tongue) underwent a hemi-glossectomy and reconstruction with a radial forearm free flap (RFFF), and received adjuvant radiotherapy. Twelve patients (67%) were diagnosed with stage IV head and neck cancer. Three were treated with radio-chemotherapy and 9 were treated surgically. Of those treated surgically, all 9 underwent a neck dissection and 6 received reconstructive surgery involving RFFF, a pectoralis major flap (PMF), a fibula free flap (FFF), an anterolateral thigh (ALT) flap, and/or a deep circumflex iliac artery (DCIA) flap. Of these 9 patients, 8 received adjuvant radiotherapy and 1 received adjuvant iodotherapy.

When radiotherapy was indicated, IMRT was used with a total dosage ranging from 60 Gy to 72 Gy (2 Gy per session for 6 weeks). The most commonly used chemotherapy regimen was cisplatin (100 mg/m^2) given intravenously during weeks 2, 4 and 6 of the radiation treatment. All 3 thyroidectomy patients received 100 mCi I-131 (Table 3).

Questionnaires

Partner relationship quality was measured with MMQ.[21,22] The MMQ is a standardized and validated questionnaire consisting of 20 items relating to marital (MMQ-M), sexual (MMQ-S) and general life satisfaction (MMQ-G), with a 9-point (0–8) scale appended to each question. Scores on MMQ-M range from 0 to 80, and scores on MMQ-S and MMQ-G range from 0 to 40. Higher scores reflect greater dissatisfaction. In this sample, Cronbach's alpha for MMQ was acceptable with 0.74 at baseline, and good with 0.90 at the 6-month follow-up.

Sexual activity was measured with an adapted version of SAQ.[23–25] The SAQ is a standardized and validated questionnaire used to assess the importance and frequency of sexual activity over time. Measurement is based on a 4–6-point Likert-type scale. Higher scores indicate either a higher perceived importance of sexual activity, ranging from 1 (very unimportant) to 6 (very important), or less frequent sexual activity, ranging from 1 (in the hospital) to 6 (not yet).

Sexual difficulties and sexual distress due to sexual difficulties were measured by means of an adapted version of SSFS.[26]

Table 3. Head and neck cancer treatment received by each patient

Patient	Age [years]	Gender	Treatment	Type	Adjuvant treatment	Type
1	56	male	surgery	tonsillectomy bilateral + SOND (zone I–III) left	radio-chemotherapy	60 Gy + cisplatinum
2	59	male	radio-chemotherapy	72 Gy + cisplatinum	none	–
3	56	male	radio-chemotherapy	70 Gy + cisplatinum	none	–
4	82	male	radiotherapy	70 Gy	none	–
5	73	male	surgery	total laryngectomy and total thyroidectomy + MRND (zone II–V) left + PMF right	radiotherapy	60 Gy
6	54	male	radio-chemotherapy	72 Gy + cisplatinum	none	–
7	71	male	surgery	tumorectomy with mandibular continuity resection en bloc + SOND (zone I–III) bilateral + FFF	radiotherapy	60 Gy
8	68	male	surgery	total parotidectomy left + SOND (zone II–III) left	radiotherapy	60 Gy
9	58	male	surgery	tumorectomy left + MRND (zone I–V) right and MRND (zone I–III) left + ALT flap left	radiotherapy	60 Gy
10	61	male	surgery	tonsillectomy and tongue base right + MRND (zone I–V) right	radiotherapy	60 Gy
11	61	male	surgery	tumorectomy left	none	–
12	65	male	surgery	total laryngectomy + MRND (zone II–V) right and selective neck dissection (zone III–IV) left + PMF right	radiotherapy	60 Gy
13	57	male	surgery	total thyroidectomy + central neck dissection (zone VI–VII) bilateral and selective neck dissection (zone II–V) right	iodotherapy	100 mCi I-131
14	46	male	surgery	total thyroidectomy + central neck dissection (zone VI–VII) bilateral and selective neck dissection (zone II–IV) left	iodotherapy	100 mCi I-131
15	54	male	surgery	total thyroidectomy + central neck dissection (zone VI–VII) bilateral and MRND (zone I–V) left	iodotherapy	100 mCi I-131
16	61	male	surgery	tumorectomy right with mandibular continuity resection en bloc + MRND (zone I–V) right + DCIA flap and ALT flap right	radiotherapy	60 Gy
17	64	male	surgery	hemi-glossectomy left + MRND (zone I–V) left + RFFF left	radio-chemotherapy	60 Gy + cisplatinum
18	73	male	surgery	total parotidectomy left + MRND (zone I–V) left + RFFF left	radiotherapy	66 Gy

SOND – supraomohyoid neck dissection; MRND – modified radical neck dissection; PMF – pectoralis major flap; FFF – fibula free flap; ALT – anterolateral thigh; DCIA – deep circumflex iliac artery; RFFF – radial forearm free flap.

The SSFS focuses on whether and to what extent patients experienced a range of impairments in sexual functioning during the past 6 months. The impairments addressed by SSFS are a lack of sexual desire (hypoactive sexual desire), erectile difficulties, and absent or delayed orgasm. Impairments in sexual functioning are rated on a 4-point Likert scale (1 – no; 2 – slight; 3 – moderate; and 4 – severe or extreme). A sexual difficulty was considered present only when the participants reported at least a moderate impairment in sexual functioning. In addition, the respondents were asked to evaluate how distressing each sexual difficulty was for them (personal sexual distress). The severity of distress was scored on a 3-point Likert scale (1 – no or mild distress; 2 – moderate distress; and 3 – severe or extreme distress). If the respondents had a score of at least 2, then distress was considered present. Respondents with a sexual difficulty and associated personal sexual distress were considered to have sexual dysfunction.

Finally, the questionnaire contained 2 questions to measure the necessity to discuss sexual problems and issues with their partner and/or a healthcare professional. Comparing the baseline and 6-month follow-up results enabled us to identify sexual issues that patients may encounter during and after treatment.

Statistical analysis

The data was analyzed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). Paired *t* tests were conducted to compare the participants' scores regarding partner relationship quality at baseline and at the 6-month follow-up. The comparison of the participants' sexual activity and sexual functioning at the same measurement time points was done using McNemar's tests. The significance level for all analyses was set at $p < 0.05$.

Results

In total, 12 patients (67%) reported a negative impact on their sexuality and/or intimacy, which means that they reported at least 1 sexual difficulty or sexual dysfunction after their head and neck cancer treatment.

Partner relationship quality

The mean scores on MMQ for the 14 patients with a partner declined significantly between baseline (31.93 ±14.31) and the follow-up (51.21 ±17.85) (t = –7.72; p < 0.000). In addition to this decline in the patients' total scores, a significant decline between baseline and the follow-up was also found in the average scores on all subscales of MMQ (MMQ-M: t = –8.21, p < 0.000; MMQ-S: t = –3.42, p = 0.005; MMQ-G: t = –5.41, p < 0.000) (Table 4).

Table 4. Partner relationship quality scores (baseline vs. follow-up) using the Maudsley marital questionnaire (MMQ)

MMQ scale/subscale	Baseline	Follow-up	Paired *t* tests	
			t	*p*-value
MMQ-M	9.21 ±5.35	17.93 ±6.83	–8.21	0.000*
MMQ-S	15.57 ±11.34	20.07 ±8.17	–3.42	0.005*
MMQ-G	7.14 ±4.67	13.21 ±7.10	–5.41	0.000*
MMQ-total	31.93 ±14.31	51.21 ±17.85	–7.72	0.000*

Data presented as mean ± standard deviation (M ±SD).
N = 14; MMQ-M – marital component; MMQ-S – sexual component; MMQ-G – general life component; *statistically significant.

Sexual activity

Thirteen patients reported to be sexually active at baseline and 14 reported sexual activity at the 6-month follow-up. While 1 patient regained sexual activity within 1 month after the completion of treatment and 1 patient after 3–6 months, most patients (n = 12) restarted solo sexual activity within 1–3 months. Most patients (n = 11) resumed partnered sexual activity within 1–3 months, while 3 of them had not resumed sexual activity with a partner after treatment at the time of the 6-month follow-up. As the results in Table 5 show, most participants found sexual activity (n = 9), kissing (n = 10) and French kissing (n = 7) important at baseline. Their reports on the importance of these activities at the 6-month follow-up were not significantly different. The number of participants that reported feeling frustration after sexual activity was significantly increased at the follow-up (n = 12) as compared to baseline (n = 6) (p = 0.031).

After treatment, 13 patients reported difficulties with kissing, 7 of whom reported mild difficulties, whereas 6 reported severe difficulties. Of these 6 patients, 3 received radiotherapy and 2 radio-chemotherapy (either as primary or adjuvant treatment), while 1 patient was only treated surgically. The issues encountered included pain, xerostomy, mucositis, and decreased tongue mobility.

Table 5. Sexual activity scores (baseline vs. follow-up) using the sexual adjustment questionnaire (SAQ)

Category	Paired *N*	Baseline	Follow-up	McNemar's tests *p*-value
Sexually active	18	13 (72)	14 (78)	1.000
Sexual activity important	13	9 (69)	10 (77)	1.000
Kissing important	13	10 (77)	11 (85)	1.000
French kissing important	12	7 (58)	7 (58)	1.000
Sexually frustrated	13	6 (46)	12 (92)	0.031*

Data presented as number (percentage) (n (%)). * statistically significant.
Note: Paired N's differ between variables, as McNemar's test requires that each participant filled out the pre- and post-measurement questionnaires.

Sexual functioning

At baseline, 5 patients reported problems with sexual desire. This number raised to 9 at the 6-month follow-up, although the increase was not statistically significant (p = 0.125) (Table 6). Of the patients with sexual desire difficulties, 3 patients at baseline and 8 patients at the follow-up reported personal distress related to their sexual difficulty, meeting the criteria for sexual dysfunction. Finally, the reports of partner distress increased from 4 to 11 between baseline and the follow-up, while the relationship distress resulting from their low sexual desire was reported by 3 patients at baseline and 12 patients at the follow-up. At the follow-up, 3 of these patients reported an extreme loss of sexual desire, 1 of whom was an 82-year-old patient and another had been previously treated for prostate cancer.

At baseline, 4 patients reported erectile difficulties, 3 of whom experienced associated personal distress. The number of patients reporting erectile difficulties (n = 6) and dysfunction (n = 5) slightly increased at the 6-month follow-up. At baseline, 2 patients reported partner distress related to the patients' erectile difficulties and 3 patients rated their erectile difficulties as distressing for their relationship. At the 6-month follow-up, the number of patients reporting partner and relationship distress increased to 9.

In total, 6 patients indicated difficulties in achieving orgasm at baseline and the same participants reported orgasm difficulties at the follow-up. Three patients noted extreme difficulties; 1 of them had undergone a radical prostatectomy in the past and another was treated with radio-chemotherapy for a larynx tumor. All 6 patients reported personal distress at baseline as well as at the 6-month follow-up. Additionally, 3 patients considered their orgasm difficulties as distressing for their partner and their relationship at baseline, and the reports

of partner (n = 7) and relationship (n = 8) distress increased at the 6-month follow-up.

One patient at baseline and 2 patients at the follow-up reported that they achieved orgasm too fast (premature ejaculation). In all cases, premature ejaculation was accompanied by personal distress, although partner distress at baseline (n = 2) and at the follow-up (n = 3), as well as relationship distress at both measurement points (n = 3), were even more frequently reported.

Only 1 patient, the oldest one participating in the study, reported that sexual intercourse was impossible at baseline. At the 6-month follow-up, 1 additional patient reported that vaginal penetration was not possible anymore (Table 6).

Table 6. Scores for sexual difficulties (baseline vs. follow-up) using the short sexual functioning scale (SSFS)

Category	Paired *N*	Baseline	Follow-up	McNemar's tests *p*-value
Desire	18	5 (28)	9 (50)	0.125
Responsive desire	16	3 (19)	6 (38)	0.250
Erection	16	4 (25)	6 (37)	0.500
Orgasm	16	6 (38)	6 (38)	1.000
Premature ejaculation	15	1 (7)	2 (13)	1.000
Penetration	15	1 (7)	2 (13)	1.000

Data presented as *n* (%).
Note: Paired *N*'s differ between variables, as McNemar's test requires that each participant filled out the pre- and post-measurement questionnaires.

Discussing sexuality and intimacy

The last 2 questions of the questionnaire aimed to explore the importance of discussing sexuality and intimacy with one's partner and/or a healthcare professional (Table 7). At baseline, 9 out of 16 patients believed it was important to discuss sexual issues with the partner. At the 6-month follow-up, this number increased to 13 patients. At baseline, 7 patients thought it was important that healthcare professionals discussed sexuality and intimacy with head and neck cancer patients. At the 6-month follow-up, this number significantly increased to 16 patients reporting that explaining the sexual implications of head and neck cancer treatment by the doctor was valuable (p = 0.004).

Table 7. Discussing sexuality and intimacy with the partner and/or a healthcare professional (baseline vs. follow-up)

Category	Paired *N*	Baseline	Follow-up	McNemar's tests *p*-value
Important to discuss with the partner	16	9 (56)	13 (81)	0.125
Important to discuss with a healthcare professional	18	7 (39)	16 (89)	0.004*

Data presented as *n* (%). * statistically significant.
Note: Paired *N*'s differ between variables, as McNemar's test requires that each participant filled out the pre- and post-measurement questionnaires.

Discussion

Prospective study

The current study is an explorative prospective pilot study specifically focusing on sexuality in the context of QoL after head and neck cancer treatment. Most head and neck cancer QoL studies use validated questionnaires, such as EORTC QLQ-H&N35.[7] These instruments are good for evaluating global QoL, i.e., pain, speech, chewing, taste, mood, and anxiety, but with only 1 or 2 questions about sexuality, they are inadequate for analyzing sexual QoL. In order to cover that part of QoL, a questionnaire combining MMQ, SAQ and SSFS was used to assess sexuality and intimacy before and after head and neck cancer treatment. The importance of a high-quality questionnaire with multidisciplinary input cannot be underestimated. By having patients fill out a baseline questionnaire at the time of diagnosis, but before treatment, we were able to make a good comparison with the sexuality issues reported at the time of the 6-month follow-up interview.

Head and neck cancer and its treatment may have a profound effect on sexuality and intimacy, and changes in sexual functioning and the relationship with one's partner can significantly influence overall QoL.[27] It is worth noting that there is a lack of research pertaining to sexuality and head and neck cancer. Most studies examine overall QoL, without a specific focus on the sexual aspects.[28,29] The sexual problems and unmet support needs of head and neck cancer patients are often not adequately identified, and until now, no real guidance or treatment options have been studied. The fact that sexual problems tend to persist after a 6-month follow-up period supports the need for better screening and treatment.[17,30]

The marital, sexual and general life satisfaction MMQ scores significantly declined between baseline and the follow-up, indicating that head and neck cancer and its treatment bring stress into the relationship. It is important to note that the effect size for the marital subscale was greater than for the sexual subscale, meaning that there was a greater decline in relationship satisfaction than in sexual satisfaction. This bigger decline in relational satisfaction may be due to the fact that in the acute phase and during the first months after treatment, patients lose their interest in sexuality and are more focused on relationship satisfaction because of stress due to the diagnosis.

As described in previous studies and as our study suggests, the levels of sexual functioning decrease after the diagnosis and treatment of head and neck cancer.[31–33] While most patients admitted that sexual activity, even at old age, remains important, kissing problems and sexual frustration were prevalent among the majority of patients after treatment. This is not surprising, considering that head and neck cancer surgery can change one's appearance and ability to kiss. Also, cancer takes a considerable

toll on daily life, especially in the case of patients that undergo radio(chemo)therapy.[34] This also became apparent when analyzing the SSFS data, suggesting that erectile dysfunction and the loss of sexual desire may become more prevalent and severe after treatment. A near doubling of patients who reported a decrease in sexual desire after treatment is a striking finding, although without reaching statistical significance. As this finding referred to a small group, it should be further studied in a larger population. Unfortunately, a comparison between patients with different cancer sites or different treatment modalities was difficult to conduct because of the small study population. However, we did notice a trend toward having more sexual problems among patients with oral cavity cancer and/or patients that underwent radio(chemo)therapy. As expected, patients with a higher tumor stage at the time of diagnosis tended to have more sexual problems as well. These results are in line with other studies in the literature.[16]

In the case of head and neck cancer there is a male predominance (2.5:1 in Western Europe) due to a higher prevalence of tobacco and alcohol abuse.[35] Interestingly, all our study patients were men. We approached 6 women to participate in the study, but unfortunately all declined. This is somewhat unexpected, as women in studies on gynecological cancers seemed quite willing to talk about these types of problems.[13] Other studies have shown that men tend to be more satisfied with their sexual QoL than women.[16]

Interventions for sexuality and intimacy

In our study, it became apparent that at the 6-month follow-up, more patients realized the importance of proper guidance in managing their sexual problems. The management of sexual problems should start even before the treatment begins. Medical specialists are obliged to provide information about a given treatment modality, including its possible complications and side effects, and they should obtain oral informed consent before a therapeutic intervention can be started. It seems that the sexual side effects of treatment are rarely discussed. It is striking that often no information is given regarding this important determinant of QoL.

Discussing sexual issues with the patient before treatment shows that the healthcare provider is aware of their importance, and it may lower the threshold to talk about possible problems in the future. It is also crucial to include the partner in the conversation.

After cancer treatment, the first step toward the optimal way of managing sexual problems is to identify patients at risk for sexual issues. The identification of patients with sexual problems could be done easily by using a screening instrument, such as SSFS. When the patient and their partner are open to therapy, treatment is typically combined with providing adequate information about the sexual side effects of interventions, and can include a combination of strengthening coping strategies, sex therapy, sexual aids, and sexual experimentation without the need for sexual intercourse. These must be discussed during follow-up consultations and tailored to what patients and their partners need.

Due to the sensitive nature of sexuality and intimacy, some healthcare providers find it difficult to talk about these issues with their patients. Research has shown that patients would prefer that their doctor initiated the discussion. The BETTER model is a mnemonic that can help to facilitate the conversation.[36] The acronym BETTER refers to: **B**ring up the topic of sexuality; **E**xplain that it is part of QoL; **T**ell the patient that information will be given; **T**ime the discussion and let the patient know that sexuality can be talked about at any point in time; **E**ducate the patient about the sexual side effects of treatment; and **R**ecord findings in the patient's medical records.[37] Specific suggestions that enable patients to improve their sexual functioning or experience (e.g., changing medication, the use of a lubricant or the use of a phosphodiesterase type 5 (PDE-5) inhibitor) are useful. The last step is referring patients with sexual problems to a qualified psychosexual therapist.[38]

Limitations

As with every questionnaire-based study, the answers given are subjective and represent a snapshot of one particular moment in time. A longer follow-up (12 months) would provide us with even more data and the ability to assess if sexual function recuperates or stabilizes with time.

Our study sample was limited to 18 patients that were gathered from different departments (i.e., maxillofacial surgery, otorhinolaryngology and radiotherapy-oncology) and it proved to be difficult for a single part-time healthcare professional to see patients at the different locations. It is also worth noting that sexuality and intimacy remains a sensitive topic, and that it may still be a big taboo for both patients and medical specialists. The aforementioned issues inherently limited the sample size of the current study.

Conclusions

The current prospective study revealed that a majority of head and neck cancer patients experience sexual problems during and after treatment. The persistence of sexual problems, as shown in our long-term follow-up study, and the fact that patients often do not realize the need for information and guidance until after treatment, makes it essential for healthcare professionals to bring up the topic of sexuality before treatment and during follow-up consultations. Effective intervention techniques for sexual problems start with the identification of patients with sexual problems by using a screening

instrument and discussing a treatment plan. However, more research must be conducted to find out how to effectively implement specific sexual intervention programs in head and cancer treatment centers. Only by bringing together the knowledge of head and neck cancer specialists and the expertise of a qualified psychosexual therapist will we be able to help patients with the sexual problems they are confronted with.

Ethics approval and consent to participate

The study protocol and procedure were approved by the Ethics Committee at the University Hospitals Leuven, Belgium (No. of approval: S-59565). Written informed consent was obtained from all participants.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Intraorally welded wing abutments supporting full-arch prostheses: A retrospective clinical study

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Abstract

Background. Delayed loading in the rehabilitation of edentulous patients with an implant-supported prosthesis implies a longer treatment time. It requires additional surgery to uncover the submerged implants, and this may increase patients' discomfort and morbidity.

Objectives. The immediate loading-based technique described in this article involves creating a metal framework by intraorally welding the implants pair by pair, using specific wing abutments. The aim of the study was to investigate the implant-prosthetic success and complication rates of this technique when used to rehabilitate totally edentulous patients.

Material and methods. The clinical records of totally edentulous patients were retrospectively evaluated. The prosthetic success rate as well as technical and biological complications were analyzed. Furthermore, the implant survival and success rates were assessed by measuring marginal bone loss (MBL) at the implant, prosthesis and patient levels.

Results. The records of 37 patients (284 implants and 43 prostheses) were included in the study. At the last follow-up (45.5 ±33.6 months), the prosthetic success rate was 100%. Mucositis affected 4 implants (1.4%), while peri-implantitis affected 4 implants (1.4%). Meanwhile, 7 implants (2.5%) showed a lack of early osseointegration. According to the criteria of Albrektsson et al., 271 implants (95.4%) were successful. The average MBL at the implant, prosthesis and patient levels was 0.26 ±0.42 mm, 0.26 ±0.19 mm and 0.26 ±0.18 mm, respectively.

Conclusions. The technique described in this article seems to be a viable approach to the rehabilitation of totally edentulous patients through immediate loading. However, these results should be confirmed by appropriately designed prospective and comparative clinical studies.

Keywords: dental implants, immediate loading, implant

Cite as
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Introduction

The rehabilitation of edentulous patients with an implant-supported prosthesis traditionally involves placing the implants, and then loading the prosthesis after 3–4 months in the mandible and 6–8 months in the maxilla.[1] This stress-free healing period allows for the osseointegration of the implant, minimizing the risk of implant failure and loss.[2] However, delayed loading implies a longer treatment time and additional surgery to uncover the submerged implants, which may increase patients' discomfort and morbidity.[2] For this reason, more recently, immediate and early loading have been studied and found to be, under certain conditions, viable options. They involve delivering the prosthesis within 48 h and between 3 and 12 weeks, respectively, after implant placement, thus reducing the time for functional rehabilitation as well as patients' discomfort and morbidity.[3] Under appropriate conditions, the success rate of the implants placed according to the immediate and early loading protocols is high and similar to that obtained with delayed loading.[2,4] Moreover, immediate loading is associated with low bone loss and good esthetic results.[2,5] Indeed, in two-stage procedures, the prosthesis is loaded after 3–4 months or up to 6–8 months after implant placement, allowing significant bone remodeling to occur during this time.[5,6] Furthermore, in the delayed loading protocol, surgical reoperations for prosthesis delivery could bring additional trauma and tissue damage, leading to a greater marginal bone loss (MBL).[6] On the contrary, in the immediate loading procedures there is no second surgery and no delay time between the 2 stages, which reduces bone stress; immediate/early mechanical loading may have a positive effect on the initial phase of healing and may stimulate the formation of the alveolar bone.[6]

The micromovements of fixtures must not exceed 100–150 μm immediately after placement to ensure success, regardless of the loading type. Indeed, osseointegration does not occur if this happens, and fibrous tissue may develop at the bone–implant interface, leading to implant failure.[7] Therefore, adequate primary stability is necessary to achieve success in implant-prosthetic rehabilitation. The success of immediately loaded implants is also affected by the design of the prosthesis, the material it is made of and the way it is connected to the implants. Metal-ceramic prostheses are currently one of the most recommended solutions for several reasons, including their high marginal precision, long duration and excellent bond with the ceramic coating, as well as the possibility to use different modeling techniques.[8] However, new interesting prosthetic materials are emerging; they show advantageous properties, especially with regard to temporary prostheses and immediate loading. For example, semi-crystalline poly-ether-ether-ketone and its various formulations have an elastic modulus that is similar to that of the cortical bone, which reduces the occlusal forces acting on the prosthesis.[8] Increasing the durability and esthetics of this material would bring an extremely interesting alternative to metal-ceramic in the near future.[8]

Another important aspect of the success of implant-prosthetic rehabilitation through immediate loading is the connection between the implants and the prosthesis. In fact, no residual tensions should exist, as these might be transferred to the bone–implant interface, jeopardizing osseointegration.[7,9] A lack of residual tension also contributes to preventing technical complications, such as screw loosening and/or breaking, as well as abutment fractures.[10,11] The implant splinting technique has proven to be useful for increasing the mechanical stability of the implants and impression precision, which is necessary to fabricate an accurately fitting prosthesis.[10,12,13] Accordingly, several authors have advocated the use of a titanium bar to connect all of the abutments, especially when addressing total edentulism. The prefabricated bar is first modeled to fit the abutments, and then welded directly inside the oral cavity.[13,14] However, this modeling procedure is challenging, since rigid cross-arch stabilization has to be obtained without inducing torsional stress to any of the implants, while adjustments made to one position may induce residual tensions elsewhere along the bar.[13] Alternative approaches involving splinting the adjacent abutments pair by pair might be equally viable in achieving appropriate stabilization while eliminating passive tensions and allowing adequate stress distribution, thus effectively addressing these practical issues. The authors have been rehabilitating fully edentulous patients using the pair-by-pair splinting approach followed by immediate loading for several years. However, they have never studied the 2 techniques systematically with respect to their clinical outcomes. Therefore, the aim of the present study was to retrospectively investigate the viability of this approach in terms of safety and implant and prosthetic success over a medium-term follow-up period while preliminarily assessing the hypothesis that both techniques (pair-by-pair splinting and immediate loading) are similar to other full-arch abutment-splinting approaches.

Objectives

This study aimed to assess the success of full-arch definitive prosthetic rehabilitation achieved using the pair-by-pair splinting and immediate loading techniques. For this purpose, technical (prosthesis unscrewing, chipping or fracture, screw loosening or fracture, welding points fracture) and biological (non-osseointegration, peri-implantitis, mucositis) complications were evaluated. The secondary objective of the study was to assess the success and survival rates of implant-supported prostheses fabricated using the technique under investigation. This was evaluated by measuring MBL and implant success based on the criteria of Buser et al.,[15] modified by Albrektsson et al.[16,17] These are as follows: the implant

should be immobile when tested clinically; the implant area should not show any signs of persistent pain, dysesthesia or paresthesia; the implant area should not be affected by peri-implant infections; and peri-implant bone resorption should be less than 1.5 mm during the first year after loading or 0.2 mm/year during the following years. Implants were considered successful when all the abovementioned conditions were met.

Material and methods

The authors retrospectively assessed the clinical data of patients who were rehabilitated for total edentulism by means of the pair-by-pair splinting technique followed by immediate loading, described in the next paragraph, between 2003 and 2020 at an Italian private dental clinic in Noventa Vicentina. The patients were followed up at the same clinic through periodic routine control visits. The study protocol was assessed and approved by the Internal Ethics Commission of the Clinic. Due to the retrospective nature of the study, the Commission did not deem it necessary to ask for an approval by a third-party Ethics Committee. The records were selected if the patient met the following criteria: aged 18–85 years; had been rehabilitated for total edentulism using the technique of interest; had had no previous surgical procedure for bone grafting or bone regeneration; had not received any peri-implant bone regeneration at implant placement; had been followed up for at least 14 months after implant placement; and had given their informed consent before treatment for the use of their clinical data for subsequent clinical studies. In addition, the clinical records had to include the intraoral radiographs collected after implant insertion and at the last follow-up visit with the use of a customized silicone bite and a Rinn centering device. The data was excluded if the patient was suffering from osteoporosis or any other diseases that affect bone metabolism, was undergoing bisphosphonate therapy, was suffering from neoplasia or a psychiatric disease, had a history of chemotherapy or radiotherapy in the head or neck region, was immunocompromised, had coagulation disorders, suffered from acute oral infections, was pregnant, was a moderate to heavy smoker (>10 cigarettes/day), had a history of drug or alcohol abuse, or was taking any drug that could interfere with the osseointegration process.

Surgical and implant placement protocol

All patients underwent a clinical examination, and radiographic assessment was performed using cone-beam computed tomography (CBCT) and panoramic X-rays. All patients were prescribed antibiotic prophylaxis (2 g amoxicillin/clavulanic acid (Augmentin®); GlaxoSmithKline, Verona, Italy) 1 h before surgery and every 12 h for 8–10 days post-surgery. In addition, postoperative care included mouth rinses with chlorhexidine 0.2% (Corsodyl®; GlaxoSmithKline) for up to 2 weeks after surgery and pain medication (500 mg naproxen sodium (Synflex®); Recordati, Milan, Italy) 2–4 times a day for 7 days after a surgical intervention.

Surgical procedures were carried out under local anesthesia using articaine hydrochloride (40 mg/mL) with epinephrine 1:100,000 (Citocartin®; Molteni Dental, Milan, Italy). After elevating a full-thickness flap, the implants were placed in the planned positions according to the manufacturer's instructions. All of the provisional and definitive prostheses were made of the same material and manufactured by the same technician. The pair-by-pair splinting technique followed by immediate loading was used to rehabilitate the patients. Intraoral radiographs were taken immediately after loading (at the provisional prosthesis delivery) and 6 months later (at the definitive prosthesis delivery), with additional radiographic assessment performed at least every 12 months thereafter. At each control visit, the prosthesis was unscrewed to provide the patient with adequate hygiene, which allowed for the assessment of implant osseointegration, and signs of peri-implantitis and mucositis.

Pair-by-pair splinting and immediate loading technique

The splinting technique involved using special abutments with protruding lateral extensions (Wings®; T.A.B., Borso del Grappa, Italy). These structures were first screwed to the implants. After that, the lateral 'wings' were cut to the desired length to partially ovelap those of the adjacent implants, and then they were welded intraorally. The resulting metal structure constituted the internal reinforcement of the provisional prosthesis, which was delivered to the patient immediately after implant surgery. The whole procedure is being marketed in Italy under the "Instantaneous Loading Technique" trademark.

In detail, the technique involves fabricating the provisional and definitive prostheses directly in the patient's mouth over a metal structure (Fig. 1). The metal support is made of several wing abutments, available in different lengths (1.7 mm, 2.7 mm and 4.5 mm), which are connected to the implants by means of 20-millimeter-long screws and welded to each other to connect the adjacent implants. The abutment lateral extensions (i.e., the wings) are 11.5 mm wide and have different angles (30°, 45°, flat). The extensions are cut to the desired length to partially overlap those of the adjacent implants, and then they are welded. The protruding parts are convex on the buccal side and flat on the vestibular side. This configuration limits the contact area between the two wings, which increases the electric resistance between the two metal pieces. During intraoral welding, specific current is applied; it leads to the release of heat at the contact point, allowing a strong weld between the two wings.

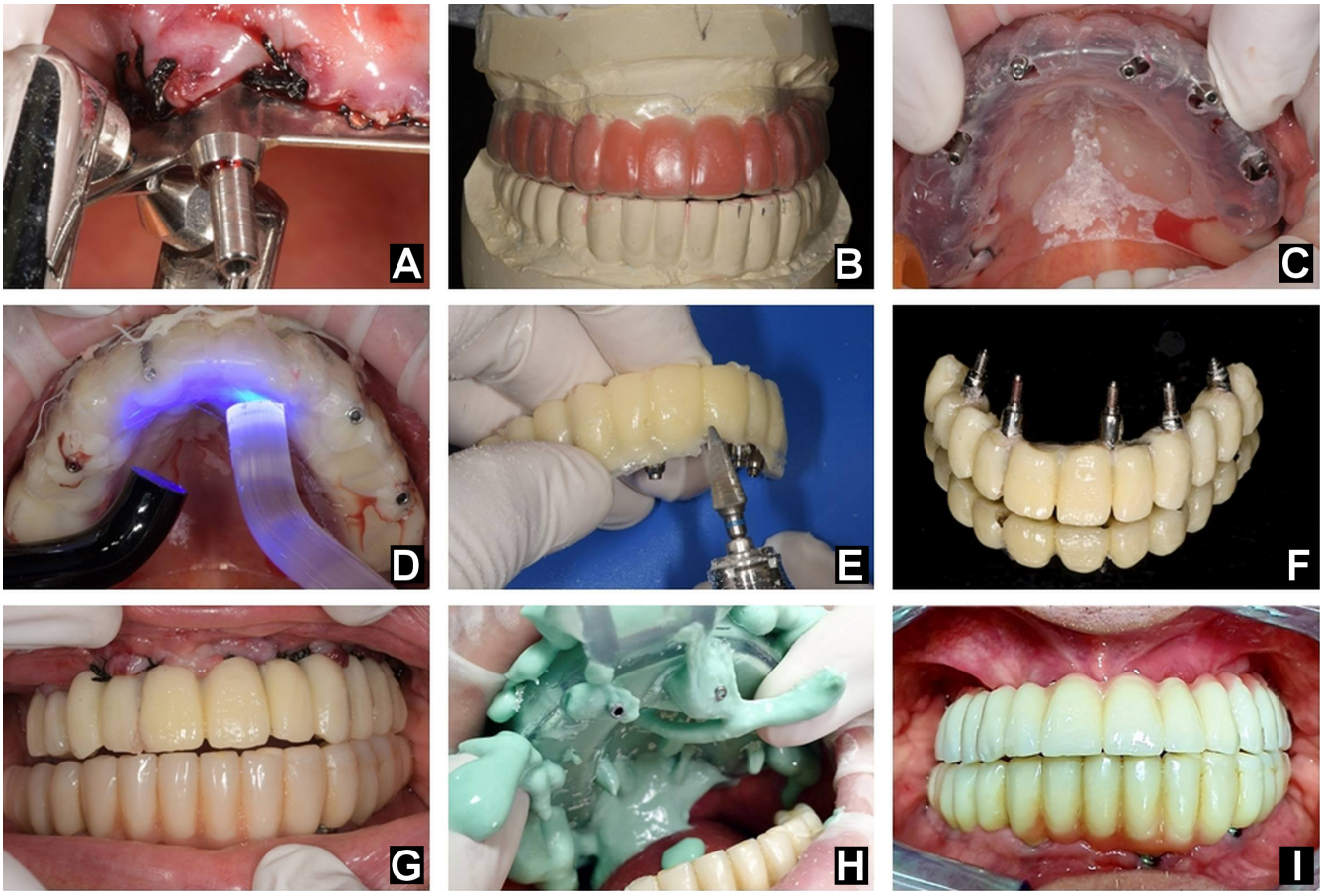

Fig. 1. The main steps of the Instantaneous Loading Technique

A – intraoral welding of the wing abutments; B,C – producing and positioning of the prosthetic thermoplastic template; D – photopolymerization of the resin; E,F – smoothening and finishing of the provisional prosthesis; G – provisional prosthesis delivered to the patient; H – alginate impression of the definitive metal framework; I – definitive prosthesis delivered to the patient.

The provisional prosthesis is delivered immediately after the metal structure is welded. First, a prosthetic thermoplastic template is shaped according to the diagnostic waxing obtained from the alginate impression of the arch of interest. Then, the template is positioned on the welded structure in the oral cavity and holes are drilled to accommodate pass-through screws. Subsequently, the template with the protruding screws is filled with a photopolymerizing resin. Once the resin has set, the prosthesis is smoothened, finished and screwed to the metal structure. The definitive prosthesis is delivered after 6 months. A new wing metal structure is created and an alginate impression is taken. Alginate was chosen for all impressions, since it is a low-cost material and it is easy to manipulate while allowing performing a detailed impression in one step. Moreover, it is well tolerated by the patient, the instrumentation is very simple and it takes a short time to prepare the impression. This is particularly important considering that the prosthesis has to be fabricated immediately after implant placement and metal structure welding. Based on this new metal structure, the technician fabricates the definitive prosthesis using a composite resin, which is supported and reinforced by the embedded new metal structure. The prosthesis is screwed onto the implants with a torque of 35 N·cm. Lastly, using the same composite resin as in the prosthesis, the screw holes are filled. Figure 2 shows a representative case.

Measurement of marginal bone loss

Marginal bone loss was measured on intraoral radiographs, after digitizing them at 600 dpi, using image analysis software (ImageJ, National Institutes of Health, Bethesda, USA; https://imagej.nih.gov/ij). The software was calibrated according to the known dimensions of the implants, with the implant diameter at the most coronal part of the implant neck used as a reference. Measurements were taken with an accuracy of 0.01 mm. The peri-implant marginal bone level (PBL) at a given time point was calculated as the average of the distance, measured at the mesial and distal sides of the implant, between the implant–abutment interface and the most apical point of the crestal bone that was in contact with the implant itself. Marginal bone loss that occurred between the 2 time points of interest was then calculated as the difference between the 2 corresponding PBL values.

In this study, MBL was calculated as the difference between PBL at the last follow-up and that at the definitive

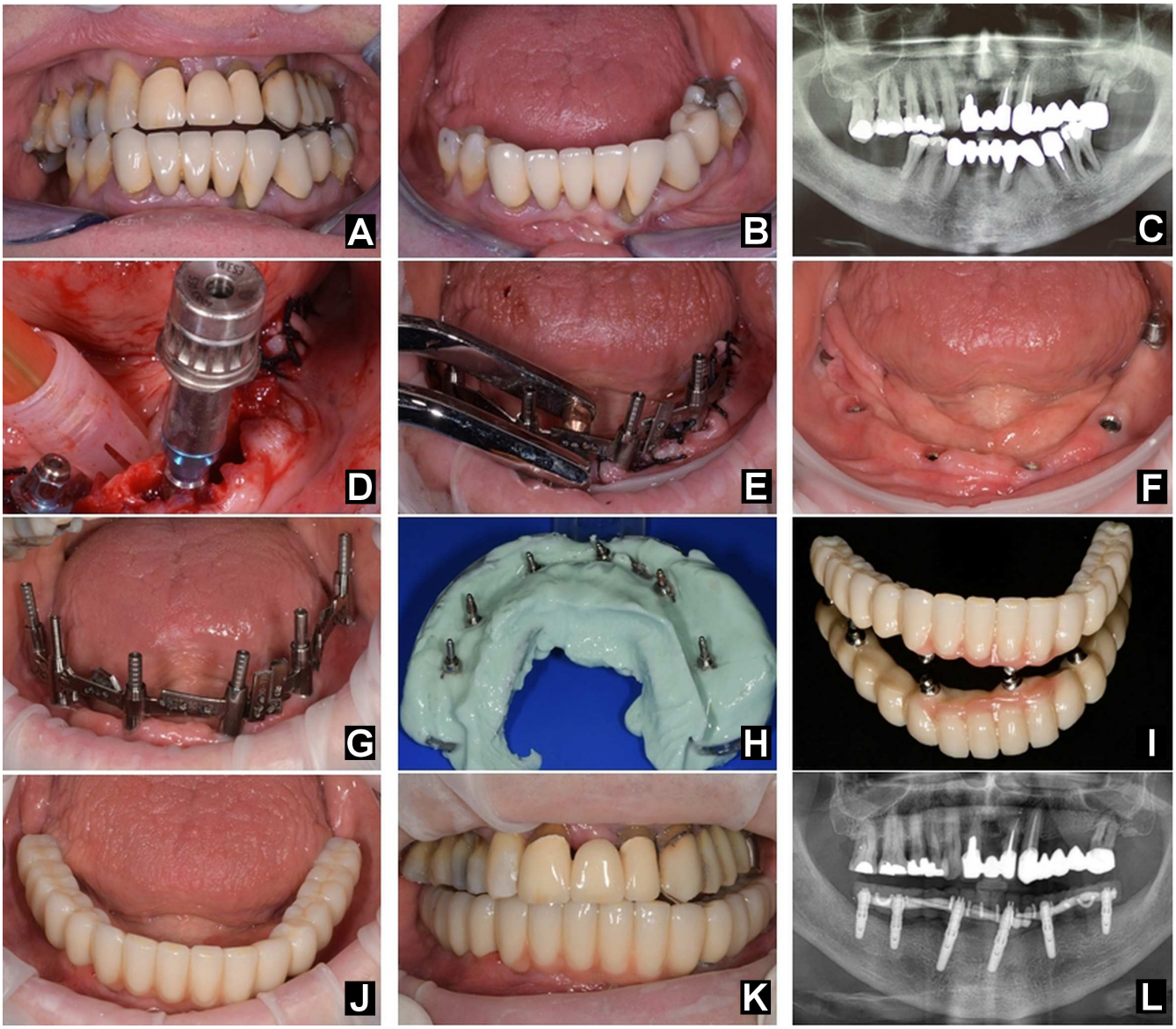

Fig. 2. Representative case

A,B – patient at clinical presentation; C – pre-surgical radiographic assessment; D – implant placement; E – welding of the wing abutments; F – soft tissue healing after 6 months; G – definitive titanium wing framework; H – alginate impression embedding the titanium structure; I – definitive prosthesis; J,K – definitive prosthesis delivered to the patient; L – orthopantomogram (OPG) after 6 months.

prosthesis delivery. This was done at the patient, implant and prosthesis levels. At the patient level, the mean MBL was calculated by averaging the MBL of all the patient's implants. At the implant level, the mean MBL was calculated by averaging all MBL values for all implants. At the prosthesis level, the mean MBL was calculated as the average of the MBL of all the implants supporting the same prosthesis.

Bias

The possible sources of bias included the fact that the retrospective analysis concerned patients treated by one of the authors (SD). Additionally, clinical records were selected and data was extracted by the 3 other authors (NZ, MM, AG). Statistical analysis was conducted by an independent biostatistician.

Statistical analysis

As the purpose of the study was limited to investigating the viability of the immediate loading technique by means of descriptive statistics, no sample size calculation was performed. Therefore, the number of clinical records considered was that of the available records that met the study inclusion criteria.

Discrete variables (the patients' gender, and the number of implants and prostheses) were summarized by means of absolute and relative frequencies (n (%)). The distribution of continuous variables (the patient's age, the follow-up time and MBL) was first tested for normality using the Shapiro–Wilk test. Then, the variables were summarized as mean and standard deviation ($M \pm SD$) or as mean and standard error of the mean ($M \pm SEM$) if normal, or as

median and interquartile range (*Me* (*IQR*)) if non-normal. As some patients underwent 2 surgical procedures for implant placement, one for each dental arch, and at different times, only the age of the patient at the 1st surgery was considered for statistical purposes. Follow-up duration was calculated both at the implant and patient level; in the latter case, the longest follow-up was considered, corresponding to the time between the last control visit and the placement of the first implant. The complication rates were calculated considering the patients, the implants and the prosthesis as statistical units. Statistical analysis was performed using standard software (Origin(Pro), v. 2022; OriginLab Corporation, Northampton, USA).

Results

Thirty-seven records concerning 16 men (43.2%) and 21 women (56.8%), 284 implants and 43 prostheses were included in the study. The mean patient age at the 1st surgery was 65.2 ±8.3 (50–85) years. As many as 84% of patients received 1 prosthesis and 16% received 2, one for each dental arch. A total of 94 implants (33.1%) were placed in the maxilla and 190 (66.9%) in the mandible. Between 4 and 9 implants were placed in each arch, and the percentages of prostheses supported by a given number of implants are reported in Table 1. Thirty-nine prostheses spanned from dental position 17 to dental position 27 in the maxilla and from dental position 37 to dental position 47 in the mandible. Four prostheses spanned from position 16 to position 26 in the maxilla and from position 36 to position 46 in the mandible. Ten prostheses were cantilevered, with the extensions spanning one element at one or both sides (Table 2).

The implants had different diameters (3.20, 3.25, 3.30, 3.70, 4.00, 4.10, and 5.00 mm) and lengths (8.50, 10.00, 11.00, 11.50, 13.00, and 15.00 mm). The models and brands were as follows: BTK® (*n* = 225; Biotec, Povolaro, Italy); Osseotite® (*n* = 31; Biomax, Vicenza, Italy); Normomix® (*n* = 14; Or-Vit Viteria Ortopedica, Castel Maggiore, Italy); and ShapeOne® (*n* = 14; iRES SAGL, Mendrisio, Switzerland). Although implants from different manufacturers were used, all were tapered and had a sandblasted, double-etched surface. The patients were followed up for a mean period of 45.5 ±33.6 months (range: 14.0–217.0; 40.0 (23.0–57.0)), and the average follow-up time for implant insertion and prosthesis loading was 45.0 ±31.9 months (range: 14.0–217.0; 44.0 (22.5–56.0)).

Table 1. Distribution of prostheses according to the number of implants they were supported by

No. of implants for each arch	No. of prostheses *n* (%)
4	1 (2.3)
5	7 (16.3)
6	12 (27.9)
7	14 (32.6)
8	6 (14.0)
9	3 (7.0)
Total	43 (100.0)

Table 2. Distribution of prostheses according to their spanning and cantilevers

Cantilevers	Spanning from 17 to 27/ from 37 to 47	Spanning from 16 to 26/ from 36 to 46	Total
Non-cantilevered	33 (76.7)	0 (0.0)	33 (76.7)
Cantilevered (one-unit extension at one side)	4 (9.3)	0 (0.0)	4 (9.3)
Cantilevered (one-unit extension at both sides)	2 (4.7)	4 (9.3)	6 (14.0)
Total	39 (90.7)	4 (9.3)	43 (100.0)

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

Outcome data

After implant placement and prosthesis loading, 1 patient (2.7%) complained of pain associated with inflammation and redness of the mucosa at 1 implant site (0.4%). Mucositis affected 1 patient (2.7%) and 4 implants (1.4%). Peri-implantitis affected 2 patients (5.4%) at 4 implants (1.4%); in 2 cases, one for each patient, 1 implant was lost (0.7%). Seven implants (2.5%) in 6 patients (16.2%) showed a lack of early osseointegration, which led to implant loss in 6 cases (2.1%) in 5 patients (13.5%). Two implants (0.7%) in 1 patient (2.7%) showed peri-implant radiolucency (Table 3).

Minor technical complications occurred in 6 prostheses (14.0%) of 3 patients (8.1%). Four cases (9.3%) involving 3 patients (8.1%) regarded a minor fracture of the composite material. Two of these (4.7%) involved the same patient (2.7%) and concerned the provisional restoration. In 2 cases (4.7%) regarding 1 patient (2.7%), the wear of the prosthesis occurred. In all cases, the defects were easily solved on the same day of the control visit and did not require the substitution of the prosthesis. Therefore, the success rate of the definitive prostheses can be considered to be 100%. No fracture of the welding points occurred. Furthermore, no screws were fractured or lost (Table 4).

Table 3. Biological complications by patient and by implant

Biological complication	Patients *N* = 37	Implants *N* =284
Pain	1 (2.7)	1 (0.4)
Mucositis	1 (2.7)	4 (1.4)
Peri-implantitis	2 (5.4)	4 (1.4)
Peri-implant radiolucency	1 (2.7)	2 (0.7)
Lack of early osseointegration	6 (16.2)	7 (2.5)
Lost implants	5 (13.5)	6 (2.1)

Data presented as *n* (%).

Table 4. Technical complications by patient and by prosthesis

Technical complication	Patients *N* = 37	Prostheses *N* =43
Prosthesis fracture	0 (0.0)	0 (0.0)
Minor fracture of the composite material	3 (8.1)	4 (9.3)
Prosthesis wear	1 (2.7)	2 (4.7)
Fracture at the welding points	0 (0.0)	0 (0.0)
Screw fracture	0 (0.0)	0 (0.0)
Unscrewing	0 (0.0)	0 (0.0)

Data presented as *n* (%).

According to the criteria of Buser et al.,[15] modified by Albrektsson et al.,[16,17] 271 implants (95.4%) were successful. Of the 13 implants that were not, 5 (1.8%) survived and 8 (2.8%) were lost (Fig. 3).

At the last follow-up, the average MBL at the implant level was 0.26 ±0.42 mm (range: 0.00–2.83), at prosthesis level it was 0.26 ±0.19 mm (range: 0.00–0.90) and at the patient level it was 0.26 ±0.18 mm (range: 0.00–0.90) (Fig. 4).

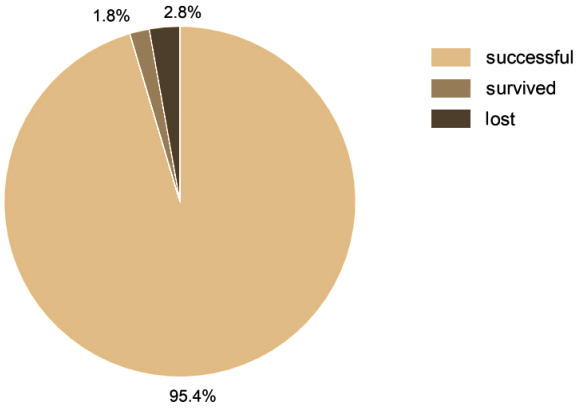

Fig. 3. Implant success at the last follow-up
According to the criteria of Albrektsson et al.[16,17]

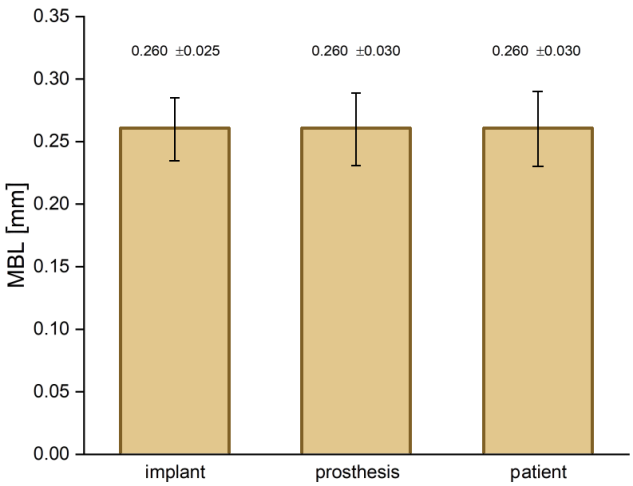

Fig. 4. Marginal bone loss (MBL) at the last follow-up, calculated at the implant, prosthesis and patient levels
Data presented as mean ± standard error of the mean (*M* ±*SEM*) for the sake of clarity.

Discussion

The results of the present study show that the technique under investigation allows the successful rehabilitation of totally edentulous patients over a medium-term follow-up period without severe complications. Almost 4 years after treatment, the implant survival and success rates were 97.2% and 95.4%, respectively. These values are similar to those reported in the literature on immediate loading. Indeed, a systematic review conducted by Del Fabbro et al. in 2019 evaluated 5,349 implants immediately loaded in 1,738 patients and reported a mean implant survival of 97.4% at a mean follow-up of 72.4 months.[18] A similar survival rate (97.7%) was previously observed by Rivaldo et al. after 18 months since loading in 33 patients with totally edentulous mandibles.[19] The rehabilitation involved a complete, immediately loaded, fixed prosthesis supported by 3 implants for each patient.[19] Primo et al. reported a 95.3% success rate in 20 completely edentulous patients at a mean follow-up of 66.8 months.[20] The patients were treated using the immediate loading, three-implant-supported prosthesis rehabilitation protocol.[20] In a comparative clinical study, Alfadda achieved similar results (96% of success) 1 year post-surgery after rehabilitating 45 patients, in whom 160 immediately loaded implants were inserted.[21]

The implant survival and success rates of the present study may appear slightly lower than those obtained in other studies using a similar abutment-splinting technique. Degidi et al. reported a 100% implant success at a 12-month follow-up examination in 20 patients who had received 153 implants that were fixed with an intraorally welded titanium bar.[22] The same authors prospectively treated 20 patients who received 80 interforaminal implants in edentulous mandibles.[23] After implant placement, the abutments were connected to the implants and welded to a titanium bar. Subsequently, the definitive prosthesis was screwed onto the metal framework. The implant survival rate was 100% at a 24-month follow-up.[23] The same survival rate was also observed by Albiero et al. 1 year after surgery in 10 consecutive patients treated with the guided-welded approach.[24] According to this protocol, the implants were inserted using computer-assisted surgery and immediately loaded with a full-arch prosthesis supported by an intraorally welded titanium structure.[24] However, even though the survival and success rates of the abovementioned studies were slightly higher than those obtained in the present study, the mean follow-up periods of these works were shorter, being limited to 12–24 months, and the results relate to fewer implants and patients. These facts may explain the apparent difference in outcome. As a whole, the present results seem to be in line with those reported in the literature.

In this study, the definitive restoration was successful in 100%. Indeed, technical complications concerned a minor superstructure fracture and the wear of the prosthesis, involving no more than 1 element, and were easily repaired

without compromising the function of the dentures. Several previous studies on immediate loading, both on implant-supported prostheses in general and on metal welded structures in particular, showed comparable results. Klee de Vasconcellos et al. reported a 100% prosthetic success in 40 patients with edentulous mandibles treated with a fixed denture supported by an intraorally welded titanium bar.[25] Similarly, Degidi et al. rehabilitated 40 patients with edentulous mandibles using a fixed restoration supported by an intraoral welded metal structure.[14] The prosthetic survival rate was 100% at a 24-month follow-up. However, 2 patients reported the fracture of the acrylic resin superstructure, though this did not compromise the function of the prostheses, which were easily repaired.[14] The prosthetic success rate was also 100% in a study by Marchesi et al., who rehabilitated 17 patients with 2 parallel and 2 tilted implants in an edentulous maxilla.[26] They were immediately loaded with fixed prostheses and were followed up for an average of 26.5 months.[26]

Soft tissue complications were observed in only 2 cases in this study and they were not of particular relevance. One patient had mild inflammation and redness of the mucosa at 1 implant site, and complained about mild pain, which resolved spontaneously, whereas another patient had mucositis at 4 implant positions. Moreover, the same patient presented radiographic radiolucency and peri-implantitis at 4 other implant sites. This patient (59 years old at the time of surgery) was a smoker (10 cigarettes/day) and had a history of periodontal diseases since youth. Considering her partially compromised oral conditions, the patient had been offered rehabilitation with a removable prosthesis, but she declined and asked for a fixed implant-supported one. She was advised about the risk, but accepted anyway, and was finally satisfied with the result.

The mean MBL observed in the present study after an average of 45 months since loading (0.26 mm) was lower than that reported by Klee de Vasconcellos et al. (1.11 mm at 18 months),[25] Degidi et al. (0.57 at 12 months)[22] and Degidi et al. (0.59 mm at 24 months).[14] This suggests that the technique under investigation allowed the creation of a precise and passively fitting structure, shaped in accordance with the anatomy of each individual patient, and did not induce stress to the underlying bone. This may favor the osseointegration of implants, and thereby the success of the implant-prosthetic rehabilitation of edentulous patients. However, a clear comparison between the MBL values observed in this study and those previously mentioned cannot be firmly sustained given the different surgical and prosthetic protocols used.

Overall, the current results suggest that splinting the adjacent implants in pairs, according to the technique under investigation, may be a good solution to achieve a passive fit between the restoration and the implants while avoiding severe biological and technical complications. This technique is easy and fast to perform, especially as the wing abutment extensions are provided with different angles, which allows the shaping of the metal frame to the specific anatomy of each patient. Furthermore, the cost of this approach is lower as compared to other rehabilitation procedures. It might therefore be a fair option, with acceptable esthetic and functional outcomes, for those who cannot afford more expensive treatment.

Limitations

The main limitation of this study is that it was retrospective. This means that the results might be biased by a number of confounding variables that could not be controlled, including the heterogeneity of the implant brands being used, the different number of implants supporting the prostheses and the presence of some cantilevered prostheses. Also, all patients were treated by the same surgeon at a single clinic. Furthermore, the present use of intraoral radiographs to assess implant success and survival is not sufficient to determine osseointegration on its own, which may limit the validity of the present results. Therefore, comparisons between the present results and those in the literature on immediate loading and similar techniques should be made with caution, and generalizing the results should be limited to some extent.

The most important advantage of the pair-by-pair system over classical approaches is the rapid element welding, which obviates the usually cumbersome need to cut and bend a bar. Prospective studies, choosing specific covariates other than the technique itself, are necessary to assess the performance and safety of this approach without bias. Comparative studies are also warranted to assess whether the technique provides any advantage over other splinting techniques associated with immediate loading.

Conclusions

Within the limitations of the present study, the immediate loading technique used by the authors seems to be a valid option for the rehabilitation of totally edentulous patients. This approach allowed high implant-prosthetic success without severe biological and technical complications. It appears to be as easy, fast, inexpensive, safe, and effective as other rehabilitation procedures involving the fixation of implants with intraorally welded metal bars. However, this should be further assessed by appropriately designed prospective and comparative clinical studies.

Ethics approval and consent to participate

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

Data availability

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

Consent for publication

Not applicable.

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Original paper

Fear and anxiety of COVID-19 in dental patients during the COVID-19 pandemic: A cross-sectional survey in Turkey

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

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Abstract

Background. The significant risk of cross-infection in dental practice has caused indecision among dental patients about whether to attend dental appointments. The coronavirus disease 2019 (COVID-19) pandemic has had a significant psychological impact on dental patients.

Objectives. The aim of this study was to evaluate the levels of and the associated factors for fear and anxiety among dental patients during the COVID-19 pandemic in Turkey.

Material and methods. A cross-sectional questionnaire-based survey consisting of 6 parts was conducted. The 6 parts were sociodemographic data, knowledge about COVID-19, information sources, the perception of COVID-19, the fear of COVID-19 scale (FCoV-19S), and the generalized anxiety disorder-7 scale (GAD-7). A total of 301 participants completed the survey.

Results. As many as 81% of the participants perceived COVID-19 as a serious disease, and 73% reported the fear of visiting their dental clinic due to the possibility of being infected with COVID-19. The participants' knowledge about COVID-19 was significantly correlated with gender, the educational status and the use of the Internet. There was a strong negative correlation between the participants' levels of knowledge and the FCoV-19S and GAD-7 scores. A significant positive correlation was observed between the FCoV-19S score and the GAD-7 score. In regression analysis, being female, perceiving COVID-19 as a serious disease, being afraid of going to the dentist, having a low knowledge score, and having a high GAD-7 score were the predictors of a high FCoV-19S score.

Conclusions. This study determined that the COVID-19 pandemic had had significant psychological effects on dental patients in Turkey. The results also underline the importance of providing more educational information to the public about the strict infection control measures taken by dental clinics against COVID-19 transmission in order to eliminate misperception.

Keywords: anxiety, fear, COVID-19 pandemic, coronavirus disease 2019 (COVID-19), dental patient

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Introduction

Coronavirus disease 2019 (COVID-19) was initially identified in Wuhan, China, in December 2019 and it spread around the world. The World Health Organization (WHO) declared COVID-19 a pandemic on March 11, 2020.[1]

The COVID-19 pandemic rapidly changed social relationships, health conditions and the routine of people's daily lives.[2] In response to the pandemic, governments introduced various measures to prevent the transmission of the disease, including social distancing, self-isolation and quarantine orders. Due to the prolonged lockdown and COVID-19-associated fear, a large proportion of the population experienced frustration, stress and irritability.[3,4] Studies have reported that the percentage of people with anxiety, depression, fear, and sleep problems increased during the COVID-19 pandemic.[5–9]

The risk of cross-infection in dental practice has been demonstrated to be remarkably high because of close physical contact between dental patients and professionals, as well as the production of aerosol during dental procedures.[10] Professional dental associations around the world have published recommendations and guidelines dental clinics should follow during the pandemic.[11] Changes in health-seeking behaviors have been reported during the COVID-19 pandemic.[12] In such an uncertain situation, patients may remain undecided about their dental appointments. Previous reports have documented that the fear of COVID-19 contributes to delays in accessing healthcare.[13,14] The delivery of dental services globally has been severely disrupted during the COVID-19 pandemic.[15] Patients' concern and fear are related to the possibility of contracting the infection and transmitting the disease to their families. All of these factors may increase the psychological stress in dental patients.

Many studies have evaluated depressive symptoms in individuals from different segments of society during the COVID-19 pandemic, such as dental practitioners, the hospital staff and students.[16–20] However, few studies have investigated dental patients' perception and mental status regarding dental visits during pandemics.[14,21,22] A study conducted during the severe acute respiratory syndrome (SARS) epidemic in Hong Kong reported that 2/3 of dental patients were worried about getting infected during dental procedures, and thus avoided dental care.[21] Another study conducted during the Middle East respiratory syndrome (MERS) outbreak showed that 26% of dental patients expressed concern regarding attending dental appointments due to the fear of contracting the disease.[22]

Since regular dental care is a critical factor in ensuring and maintaining adequate dental and periodontal health, a better understanding of a patient's psychological state and perception during the COVID-19 pandemic would help us develop strategies for optimal dental practices. Therefore, the aim of this study was to evaluate the knowledge status and perception regarding COVID-19, and to determine the fear and anxiety levels of dental patients in Turkey during the pandemic.

Material and methods

Study design

This cross-sectional study was approved by the Ethics Committee at the Faculty of Medicine of Akdeniz University, Antalya, Turkey (No. of approval: 70904504/706), and conducted in accordance with the Declaration of Helsinki. The study followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines.[23]

Data was collected from the dental patients of the Akdeniz University Faculty of Dentistry between September 23 and October 23, 2020, after dental clinics were reopened to the public in Turkey. The inclusion criteria for participation were as follows: willingness to participate and provide informed consent; age of 18 years or above; and the ability to understand, read and write in the Turkish language. Individuals who did not consent to participate in the survey, patients who were under the age of 18 years and foreign patients who could not communicate in Turkish were excluded from the study.

The questionnaire (paper-based survey) was structured into 6 domains: general sociodemographic data (Appendix 1); knowledge about COVID-19 (Appendix 2); COVID-19 information sources (Appendix 3); the perception of COVID-19 (Appendix 4); the fear of COVID-19 scale (FCoV-19S)[24] (Appendix 5); and the generalized anxiety disorder-7 scale (GAD-7)[25] (Appendix 6) (The questionnaires as Appendices are available from the corresponding author on reasonable request.).

Measurement

Level of knowledge about COVID-19

Based on the information gathered from the published literature,[26] the questions asked to assess the participants' level of knowledge about COVID-19 regarded the transmission routes, prevention and clinical symptoms of COVID-19. Two questions were answered as either 'true' or 'false', and the remaining 2 were multiple-choice questions. Each correct answer scored 1 and the knowledge scores were calculated by summing the number of correct answers. The total knowledge score ranged from 0 to 10. Due to the lack of a standardized tool in the literature, the participants with an overall score >50% were considered knowledgeable. The Cronbach's alpha coefficient of the knowledge questionnaire was $\alpha = 0.78$.

Perception of COVID-19

Perception was assessed through the following 2 questions: "Is COVID-19 a serious disease?"; and "Are you afraid to visit the dental clinic due to the possibility of contagion with COVID-19?". Similar questions have been used in previous studies.[27,28]

Fear of COVID-19 scale

The participants' fear levels were measured with the Turkish validated version of the FCoV-19S.[29] This scale is reliable and valid for determining the fear of COVID-19 in the general population. It is a unidimensional scale with 7 questions. The score for each question is rated on a 5-point Likert-type scale ranging from 'strongly disagree' (1) to 'strongly agree' (5). To obtain the FCoV-19S score, all items were summed; the total score ranged from 7 to 35. The higher the score, the greater the level of fear of COVID-19. The internal consistency of the Turkish version of the scale was found to be 0.84.[29] The Cronbach's alpha value of this scale was found to be $\alpha = 0.80$.

Generalized anxiety disorder-7 scale

The participants' anxiety levels were evaluated using the Turkish version of GAD-7. The GAD-7 is a 7-item and 4-point Likert-type scale (0 – not at all; 1 – several days; 2 – more than half of the days; and 3 – almost every day) developed by Spitzer et al. that is used for the diagnosis, monitoring and assessing the severity of anxiety disorders.[25] The total score was calculated and the anxiety levels were classified into 4 categories (minimal: 0–4; mild: 5–9; moderate: 10–14; and severe: 15–21).[25] The GAD-7 was first adapted into the Turkish language by Konkan et al., and the Cronbach's alpha value was $\alpha = 0.85$.[30] The Cronbach's alpha value for the present scale was found to be $\alpha = 0.84$.

Statistical analysis

Descriptive values, including median (*Me*), mean and standard deviation ($M \pm SD$), and absolute and relative frequencies (n (%)), were calculated to examine the participants' characteristics. The normality of quantitative data was determined using the Kolmogorov–Smirnov test. The χ^2 test was used to determine differences between categorical variables. Differences between continuous variables were examined using the Mann–Whitney U test. Correlations were determined using Spearman's rank correlation coefficient (r). Linear regression analysis was performed to predict the potential influencing factors related to the fear of COVID-19. The regression coefficient (β) and 95% confidence interval (*CI*) were reported. All calculations were considered statistically significant at $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics for Windows, v. 23.0 (IBM Corp., Armonk, USA).

Results

Participants' sociodemographic data

A total of 301 participants completed the questionnaire (117 males and 184 females). The demographic data of the participants are summarized in Table 1.

COVID-19 knowledge

The mean COVID-19 knowledge score was 7.66 ±2.04 (*Me*: 8; range: 0–10). Eighty-seven percent of the study participants had sufficient COVID-19 knowledge. Significant differences were observed in the disease knowledge scores according to gender, where males had higher scores than females ($p < 0.05$), and the educational status, where participants that held a master's degree had higher scores than those who did not ($p = 0.001$). Furthermore, highly educated respondents (i.e., university education, a master's degree or Ph.D.) had higher knowledge scores than participants with only primary or high school education ($p < 0.001$). Among the studied demographic characteristics, gender ($r = 0.123$; $p < 0.05$) and the educational status ($r = 0.207$; $p < 0.001$) were significantly correlated with the mean knowledge scores. Furthermore, there was a positive correlation between the COVID-19 knowledge score and use of the Internet ($r = 0.133$; $p < 0.05$).

COVID-19 perception

Regarding the attitude of the participants toward COVID-19, 81% perceived COVID-19 as a serious disease. Moreover, 73% of the participants (among them, males: $n = 76$, 35%; females: $n = 143$, 65%) were afraid of going to the dental clinic due to the possibility of contracting COVID-19.

COVID-19 information sources

The source of information about COVID-19 was primarily television ($n = 231$, 77%), the Internet ($n = 206$, 68%; the official website of the Turkish Ministry of Health – 89%) and social media ($n = 168$, 56%). Among the social media resources, 38% of the participants used Twitter, 30% used Instagram, 25% used Facebook, and 7% used WhatsApp.

Fear of COVID-19

The mean FCoV-19S score of the participants was 18.10 ±5.64 (*Me*: 18; range: 7–35). The highest mean FCoV-19S scores referred to items "I am most afraid of COVID-19" and "It makes me uncomfortable to think about COVID-19", with values of 3.56 ±1.24 and 3.38 ±1.32, respectively. The lowest mean FCoV-19S score was observed for item "I cannot sleep, because I am worrying

Table 1. Participants' characteristics, and the comparison of the fear of COVID-19 scale (FCoV-19S) scores, the generalized anxiety disorder-7 scale (GAD-7) scores and the knowledge levels

Variable		*n* (%) total *N* = 301	FCoV-19S score *M* ±*SD*	*p*-value	GAD-7 score *M* ±*SD*	*p*-value	Knowledge level	*p*-value
Age [years]	18–39	201 (66.8)	18.07 ±5.71	0.677	3.43 ±4.09	0.373	1.92 ±0.49	0.985
	40–59	92 (30.6)	17.97 ±5.37		2.73 ±3.71		1.91 ±0.55	
	≥60	8 (2.7)	20.38 ±7.46		3.21 ±2.38		1.93 ±0.49	
Gender	male	117 (38.9)	16.47 ±5.10	0.000**	2.51 ±3.50	0.007*	7.97 ±2.11	0.009*
	female	184 (61.1)	19.14 ±6.05		3.65 ±4.15		7.46 ±1.97	
Comorbidity	no	259 (86.0)	18.00 ±5.56	0.450	3.17 ±3.96	0.699	7.67 ±2.08	0.832
	yes	42 (14.0)	18.71 ±6.12		3.43 ±3.89		7.59 ±1.78	
Tobacco use (current)	yes	77 (25.6)	19.03 ±6.21	0.096	4.06 ±4.89	0.027*	7.39 ±2.37	0.171
	no	224 (74.4)	17.79 ±5.41		2.92 ±3.53		7.75 ±1.90	
Marital status	married	152 (50.5)	18.09 ±5.61	0.973	2.54 ±3.29	0.028*	7.48 ±2.20	0.114
	single	149 (49.5)	18.11 ±5.68		3.89 ±4.43		7.85 ±1.85	
Having children	yes	151 (50.2)	18.01 ±5.79	0.782	2.88 ±3.76	0.148	7.49 ±2.15	0.140
	no	150 (49.8)	18.19 ±5.49		3.54 ±4.11		7.83 ±1.91	
Employment status	unemployed	181 (60.1)	18.59 ±5.57	0.068	3.08 ±3.79	0.477	7.64 ±2.01	0.806
	working/studying	120 (39.9)	17.38 ±5.67		3.41 ±4.18		7.70 ±2.09	
Educational status	primary school	37 (12.3)	18.43 ±5.30	0.476	2.32 ±3.66	0.152	6.83 ±2.43	0.009*
	high school	83 (27.6)	18.88 ±5.82		3.24 ±4.11		7.32 ±2.22	
	college	28 (9.3)	16.82 ±5.50		2.07 ±2.91		7.85 ±1.75	
	university	136 (45.2)	17.87 ±5.61		3.51 ±4.03		7.99 ±1.76	
	master's degree/Ph.D.	17 (5.6)	17.59 ±5.91		4.47 ±4.15		8.17 ±2.09	
Place of residence	urban	287 (95.3)	18.15 ±5.63	0.484	3.12 ±3.83	0.071	7.66 ±2.02	0.862
	rural	14 (4.7)	17.07 ±5.74		5.07 ±5.66		7.57 ±2.44	
Anxiety (GAD-7)	minimal	218 (72.4)	17.52 ±5.38	0.000**	–	–	7.75 ±1.98	0.080
	mild	58 (19.3)	18.31±6.00		–		7.70 ±2.21	
	moderate-to-severe	25 (8.3)	22.72 ±4.93		–		6.80 ±1.97	

COVID-19 – coronavirus disease 2019; *M* – mean; *SD* – standard deviation; * statistically significant ($p < 0.05$); ** statistically significant ($p < 0.001$).

about getting COVID-19", with a value of 1.71 ±0.99 (Table 2). The fear scores of females were significantly higher than those of males ($p = 0.028$). However, age, the marital status, having children, the employment status, the educational status, the region of residence, and having a systemic disease did not significantly affect the total FCoV-19S score ($p > 0.05$). There was a strong negative correlation between the FCoV-19S score and the COVID-19 knowledge level ($r = -0.808$; $p < 0.001$) (Table 3). There was no significant correlation between the FCoV-19S score and the sources of information ($p > 0.05$).

Participants who considered the disease to be serious had significantly higher FCoV-19S scores (18.80 ±5.65) than those who considered the disease not to be serious (15.40 ±4.06) ($p = 0.032$). Similarly, participants who were afraid to visit the dental clinic due to the possibility of getting infected with COVID-19 had significantly higher FCoV-19S scores (19.66 ±5.48) than those who were not afraid to visit the dental clinic (13.91 ±4.87) ($p = 0.040$). This fear was significantly higher in females than in males ($p = 0.017$).

Multiple linear regression analysis showed that being female ($\beta = 0.880$, 95% *CI*: 0.016 to 0.275; $p = 0.028$), having a low total knowledge score ($\beta = -0.510$, 95% *CI*: −0.935 to −0.680; $p = 0.000$), perceiving COVID-19 as a serious disease ($\beta = 0.114$, 95% *CI*: 0.058 to 0.328; $p = 0.005$), being afraid of attending a dental visit ($\beta = 0.359$, 95% *CI*: 0.504 to 0.792; $p = 0.000$), and having a high total GAD-7 score ($\beta = 0.165$, 95% *CI*: 0.123 to 0.349; $p = 0.000$) were the predictors of having a greater fear of COVID-19 (Table 3).

COVID-19 and anxiety

The mean total GAD-7 score of the participants was 4.61 ±4.45. Of all the participants, 218 (72%) had minimal depressive symptoms, 58 (19%) had mild symptoms and 25 (8%) had moderate-to-severe symptoms. Subjects with moderate-to-severe depressive symptoms were mostly female ($n = 19$, 10%), single ($n = 16$, 11%) and they were smokers ($n = 12$, 16%). There were no significant

Table 2. Scores of the participants' responses to the fear of COVID-19 scale (FCoV-19S) items

Item	Sum	*M* ±*SD*
1. I am most afraid of COVID-19	1,071	3.56 ±1.24
2. It makes me uncomfortable to think about COVID-19	1,016	3.38 ±1.32
3. My hands become clammy when I think about COVID-19	580	1.93 ±1.13
4. I am afraid of losing my life because of COVID-19	851	2.83 ±1.38
5. When I watch the news and stories about COVID-19 on social media, I become nervous or anxious	861	2.86 ±1.24
6. I cannot sleep, because I am worrying about getting COVID-19	515	1.71 ±0.99
7. My heart races or palpitates when I think about getting COVID-19	555	1.84 ±1.07

differences across the different age and educational status categories (Table 1). There was a strong negative correlation between the GAD-7 score and the COVID-19 knowledge level ($r = -0.136$; $p < 0.05$). In contrast, a significant positive correlation was observed between the FCoV-19S score and the GAD-7 score ($r = 0.236$; $p < 0.001$) (Table 3).

Discussion

This study is the first to evaluate the impact of the COVID-19 pandemic on the psychological status of dental patients in Turkey. The most important finding of this study is that the participants who were very worried about contracting COVID-19 were also identified as fearful and anxious. Given the novelty of the disease, it is natural that people are more prone to fear when faced with unknown situations.

In this study, the mean FCoV19S score was in line with the scores reported in the studies conducted in other populations.[31,32] According to our results, female gender was significantly related to increased fear.[33] The authors of a large-scale nationwide survey examining psychological distress among Chinese people during the COVID-19 pandemic observed the same gender effect, with females reporting significantly higher psychological distress than males.[34]

Perceiving the disease as serious and being afraid of attending a dental visit due to the possibility of contracting COVID-19 were found to be significant factors for the fear of COVID-19 in this study. This is an important finding that demonstrates the negative impact of the COVID-19 pandemic on the frequency of dental clinic visits. Similarly, a retrospective clinical study conducted in China reported that the COVID-19 pandemic significantly affected individuals' dental care-seeking behaviors.[35] Moffat et al. conducted a study in the United States on the identification of dental patients' perception of risk and attitude toward COVID-19; the participants reported that contracting COVID-19 from other patients in a dental clinic represented the greatest risk related to dental care.[27] Moreover, Kranz et al. reported that half of their participants delayed their dental visits and treatment due to the fear of contracting the virus during dental procedures.[14]

In this study, female participants had a significantly higher level of fear regarding dental visits due to the possibility of contracting COVID-19 as compared to males ($p < 0.05$). These results are consistent with a previous study, which found that females were more concerned about the risk of the aerosolized spread of infection during dental treatment and showed increased levels of stress during dental treatment.[22]

The majority of the participants (87%) in the present study showed a sufficient level of knowledge about COVID-19, which is essential to limiting the spread of the disease.[36] Moreover, the knowledge levels were significantly higher in patients with a higher educational status and in males ($p < 0.05$). Similarly, Zhong et al. found that young females with a low educational status tended to have less knowledge regarding COVID-19.[37] In contrast, Nooh et al. reported no significant association between individuals' COVID-19 knowledge levels and gender or educational status.[38] Despite contradictory results in the literature, the findings of this study suggest that highly educated individuals are effective in retrieving reliable information, which may influence their COVID-19 knowledge levels.

Another important finding of this study is a significant negative correlation between the participants' knowledge level and FCoV-19S score ($r = -0.808$; $p < 0.05$)

Table 3. Results of multiple linear regression analysis of the predictors of the fear of COVID-19 (with reference to FCoV-19S)

Predictors	*r*	*β*	95% *CI* for *β*	*p*-value
Gender[a]	0.145	0.880	(0.016 to 0.275)	0.028*
Knowledge level[b]	–0.808	–0.510	(–0.935 to –0.680)	0.000**
Is COVID-19 a serious disease?[c]	0.193	0.114	(0.058 to 0.328)	0.005*
Are you afraid to visit the dental clinic due to the possibility of contagion with COVID-19?[d]	0.648	0.359	(0.504 to 0.792)	0.000**
GAD-7[e]	0.236	0.165	(0.123 to 0.349)	0.000**

r – Spearman's rank correlation coefficient; *β* – regression coefficient; *CI* – confidence interval; [a] males as the reference group; [b] total score for the knowledge level; [c] response to item "Is COVID-19 a serious disease?" with 'no' as the reference group; [d] response to item "Are you afraid to visit the dental clinic due to the possibility of contagion with COVID-19?" with 'no' as the reference group; [e] total score on GAD-7; * statistically significant ($p < 0.05$); ** statistically significant ($p < 0.001$).

and anxiety level ($r = -0.136$; $p < 0.05$). These results are consistent with previous research, which determined that receiving adequate knowledge about the transmission routes of COVID-19 was correlated with lower levels of anxiety.[39]

The present study demonstrated that the COVID-19 pandemic had impacted the anxiety levels in dental patients, with 8% of them reporting moderate-to-severe anxiety, which is similar to the 7% reported among the general population in China.[40] Furthermore, Cotrin et al. reported that the COVID-19 pandemic and quarantine protocols influenced orthodontic patients' anxiety levels.[41] In the present study, there was a significant positive association between the FCoV-19S score and the GAD-7 score. Recent studies found that the fear of COVID-19 was significantly correlated with anxiety and depression.[42,43] These results suggest that dental patients may need psychological support during the COVID-19 pandemic to maintain their mental health. In addition, the current situation may affect the oral health of people in the near future, as previous studies have clearly shown a strong relationship between oral health and mood conditions, such as stress, anxiety and depression.[44]

In the present study, the major source of information associated with COVID-19 was television, followed by the Internet and social media. A cross-sectional study conducted with 9,796 respondents from the Netherlands, Germany and Italy found that most participants acquired information about COVID-19 from traditional sources (e.g., television and newspapers).[45] Moreover, there was a significant positive correlation between the participants' knowledge levels and the use of the Internet ($r = 0.133$; $p < 0.05$) observed in the present study. Our findings are consistent with previous studies.[45,46]

When evaluating the reliability and quality of the information found on the Internet, Hernández-García and Giménez-Júlvez reported that official health organizations provided more accurate information about protective measures against COVID-19.[45] In the present study, the Turkish Ministry of Health official website was the most common source of information about COVID-19 reported by the participants (89%). This website has been working efficiently since the beginning of the pandemic and its portal regularly updates information about COVID-19, according to the WHO guidelines.

Limitations

This study has several limitations. Firstly, the study was cross-sectional in nature and could not establish causality for the outcome. Additionally, the results may only reflect the mental health status during the pandemic. Secondly, although the measurements used in the study had satisfactory psychometric properties, the results were self-reported, which could have led to recall bias. However, a paper-based questionnaire was used in this study, which could have eliminated the selection bias observed in online surveys. Finally, the results were based on a single institution; nevertheless, our faculty was the only referral center for dental care in our region during the pandemic. Larger prospective nationwide studies are needed. The findings of this study are useful for public health professionals in recognizing target populations for specific COVID-19 pandemic-related mental health management and intervention strategies.

Conclusions

The results of this study suggest that the COVID-19 pandemic has had a significant psychological impact on dental patients. Psychological support may be needed to help dental patients manage their fear and anxiety. It is also understandable that, due to the increasing fear of COVID-19, patients may develop dental care avoidance behaviors. Therefore, effective physician–patient communication should be established to prevent misconception, and strict infection control measures should be taken by dental clinics to reduce the risk of COVID-19.

Ethics approval and consent to participate

The study was approved by the Ethics Committee at the Faculty of Medicine of Akdeniz University, Antalya, Turkey (No. of approval: 70904504/706). Written informed consent was obtained from all participants.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Estimation of the risk of COVID-19 transmission through aerosol-generating procedures

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Abstract

Background. The outbreak of the coronavirus disease 2019 (COVID-19) pandemic was associated with the provision of multiple guidelines for the dental profession. All elective procedures were restricted, and only emergency procedures were performed. There was fear and anxiety among dentists while performing aerosol-generating procedures (AGPs), as they were considered to pose a high risk of COVID-19 transmission.

Objectives. The aim of this study was to assess the risk of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during AGPs, and to examine the association between risk severity and the number of AGPs performed per day. The efficacy of personal protective equipment (PPE) was also assessed.

Material and methods. This cross-sectional cohort study was based on an online questionnaire form completed by 629 general and specialized dentists between January 1 and February 28, 2021. The collected data referred to the sources of COVID-19 infection, the type of PPE used and the number of AGPs performed each day by dental healthcare professionals (DHCPs). For each question, the absolute numbers of responses as well as percentages were calculated.

Results. Among the 629 DHCPs, 113 (17.97%) contracted COVID-19. The risk of contracting COVID-19 during AGPs was the same as in the case of non-AGPs, and the infection risk was not associated with the number of AGPs performed per day. The efficacy of a surgical mask with a face shield/eye goggles was higher in comparison with all other types of PPE. Differences in the infection risk across the different types of PPE used were statistically significant ($p < 0.001$).

Conclusions. The risk of COVID-19 transmission during AGPs is the same as in the case of non-AGPs. Thus, restrictions on the performance of elective AGPs should be lifted. On the other hand, the best protection during AGPs is provided by a surgical mask with a face shield/eye goggles.

Keywords: personal protective equipment, aerosol-generating procedures, COVID-19 transmission, dental healthcare professionals

Introduction

Coronavirus disease 2019 (COVID-19) was declared a pandemic by the World Health Organization (WHO) on March 11, 2020.[1] This disease is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[2] It mainly affects the respiratory system, with symptoms varying from mild flu to severe pneumonia, which can ultimately lead to respiratory collapse.[2] Other symptoms include the loss of taste and smell, headache, diarrhea, a sore throat, shortness of breath, and myalgia.[2] As far as oral manifestations of COVID-19 are concerned, xerostomia seems to be the most prevalent, followed by oral and dental pain, ulcerations, pain in the jaw bones and joints, and halitosis. Some individuals experience 2 or more oral symptoms at one time.[2] Global efforts were made to formulate a vaccine that would combat the virus, and prevent and contain the spread of COVID-19.[3] Although vaccines are one of the most successful interventions to fight viral infections, they may be associated with some adverse effects.[3] These include fatigue, redness at the injection site, limb pain, malaise, headache, muscle and joint pain, fever, and chills.[3] As far as facial and oral manifestations are concerned, there is no significant association with COVID-19 vaccination.[4]

The SARS-CoV-2 virus underwent numerous mutations over time, resulting in various strains, such as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529).[5] Further studies are needed to establish transmissibility, symptoms and treatment for the Omicron variant.[6] The efficacy of vaccines is being studied, with lesser efficacy found against the Beta and Delta variants. Booster doses are being recommended to improve the protective efficacy of vaccines against Omicron and other variants. A number of treatment options are available for COVID-19, including plasma therapy, and antiviral therapy using remdesivir, hydroxychloroquine (with or without azithromycin) and lopinavir-ritonavir. Additional therapeutics include anti-fibrotics, anti-inflammatory cytokines, such as interleukin-6 receptor antagonists and complement antagonists, anti-infectives, non-steroidal anti-inflammatory drugs (NSAIDS), systemic corticosteroids, bronchodilators, and vasodilators.[7] It has been demonstrated that azithromycin, a macrolide, provides antiviral and immunomodulatory effects in patients infected with SARS-CoV-2.[8]

The Centers for Disease Control and Prevention (CDC) has reported respiratory droplets, aerosols and close contact (less than 1 m) as major modes of transmission.[9] A droplet can be transmitted by coughing, sneezing, speaking, singing, and via contact with the mucous membranes, especially of the nose, the eyes or the mouth. Droplets >5–10 μm in diameter or fomites in the immediate environment of the infected person can carry the virus.[10,11] Airborne transmission occurs through droplets <5 μm in diameter, which develop from the evaporation of larger droplets. They remain in the air for a longer period and can travel a distance of more than 1 m.[12] Procedures that produce aerosols can also lead to airborne transmission.[13] Aerosols refer to small particles (<5–10 μm in diameter) that are capable of travelling longer distances, and thus can be easily inhaled. However, larger droplets (>20 μm in diameter) fall under the influence of gravity and are too large to be inhaled. Intermediate droplets (10–20 μm in diameter) may either remain suspended in the air or settle down.[14–16] As a result, most dental procedures generating aerosols pose a risk of transmitting COVID-19.[17] Dental procedures that generate aerosols include ultrasonic scaling, tooth polishing, air polishing, air abrasion, the use of slow- and high-speed rotary instrumentation, the use of an air-water triple syringe,[18] and intraoral radiographs.[19] Furthermore, saliva contains high levels of SARS-CoV-2, which peak in the first week of infection and can easily contribute to the spread of the disease.[10] At the beginning of the outbreak, there was no confirmed information on the transmission routes for SARS-CoV-2, and many healthcare workers were concerned about infecting their families and themselves.[20] This created severe psychological stress, which indirectly affected the quality of treatment provided.[20]

More research is needed, however, to confirm the transmission routes for SARS-CoV-2 and to measure the risk of COVID-19 infection among dental healthcare professionals (DHCPs) who perform aerosol-generating procedures (AGPs). Therefore, the current study was conducted to establish whether AGPs increase the risk of SARS-CoV-2 transmission as well as to evaluate the efficacy of personal protective equipment (PPE) in order to alleviate the concerns and anxiety of DHCPs.

Material and methods

This cross-sectional cohort study was carried out between January 1 and February 28, 2021. The study was conducted using an online questionnaire form that was sent to DHCPs via electronic mail. The targeted DHCPs included general and specialized dentists working at 12 dental colleges and hospitals located in different provinces of Pakistan. Different regions of the country were sampled, as the number of people infected with COVID-19 varied across the provinces. The e-mail addresses of DHCPs were obtained from the human resources departments of dental colleges and hospitals. An invitation to participate in the study, along with a link to the questionnaire, was e-mailed to 1,000 DHCPs. Those who consented to participate were included in the study, resulting in 629 respondents in total. A total of 371 DHCPs did not respond to the initial e-mail. Ethics approval for the study was granted by the institutional Research and Ethics Committee at the Rashid Latif Medical Complex, Lahore, Pakistan (No. of approval: RLDC/006080/20).

Table 1 shows the questionnaire, which was validated by conducting a pilot study, where 52 DHCPs at our institution answered the questions; the collected data was analyzed, and its internal consistency was evaluated by using Cronbach's alpha, which was 0.85.

After the validation of the questionnaire, the data was collected. Data analysis was performed using Microsoft Excel® 2021 for Mac, v. 16.56 (Microsoft Corporation, Redmond, USA), and the statistical software package Stata 14 (StataCorp, College Station, USA). For each question, the absolute numbers of responses as well as percentages were calculated. The analysis of variance (ANOVA) tests were used to determine the efficacy of various types of PPE, and to assess the relationship between the number of AGPs performed per day and the risk of contracting COVID-19. The significance level was set at $p < 0.05$.

Table 1. Questionnaire form

Questions	Answer options
1. Age [years]	–
2. Gender	male female
3. Institution/hospital name	–
4. How many AGPs do you perform per day?	1–3 4–5 6–7 >7
5. Did you contract COVID-19 since the outbreak of the pandemic?	yes no
6. If you contracted COVID-19, what was the source of the infection?	acquired during AGP acquired during non-AGP not sure about the source not applicable
7. Which of the following PPE items do you use when performing AGPs?	gloves, a surgical mask, a gown (disposable/surgical) gloves, a surgical mask, a face shield/eye goggles, a gown (disposable/surgical) gloves, an N95 respirator, a gown (disposable/surgical) gloves, an N95 respirator, a face shield/eye goggles, a gown (disposable/surgical)

AGP – aerosol-generating procedure; COVID-19 – coronavirus disease 2019; PPE – personal protective equipment.

Results

The number of DHCPs who contracted COVID-19 (n = 113) accounted for 17.97% of the 629 participants. Among the 113 DHCPs who contracted COVID-19, 51 (45.13%) were male and 62 (54.87%) were female. The mean age of the participants was 43 ±9.63 years. The number of AGPs performed in a day by all DHCPs as well as the calculation of the infection risk are presented in Table 2.

The ANOVA tests showed no association between the number of AGPs performed per day and the risk of contracting COVID-19 ($p > 0.05$).

A total of 39 (6.20%) of the 629 DHCPs indicated that they acquired COVID-19 through AGPs, 37 (5.88%) through non-AGPs, and 37 (5.88%) did not know the origin of the infection (i.e., were unsure). Figure 1 illustrates the percentage of DHCPs who contracted COVID-19, together with the source of the infection.

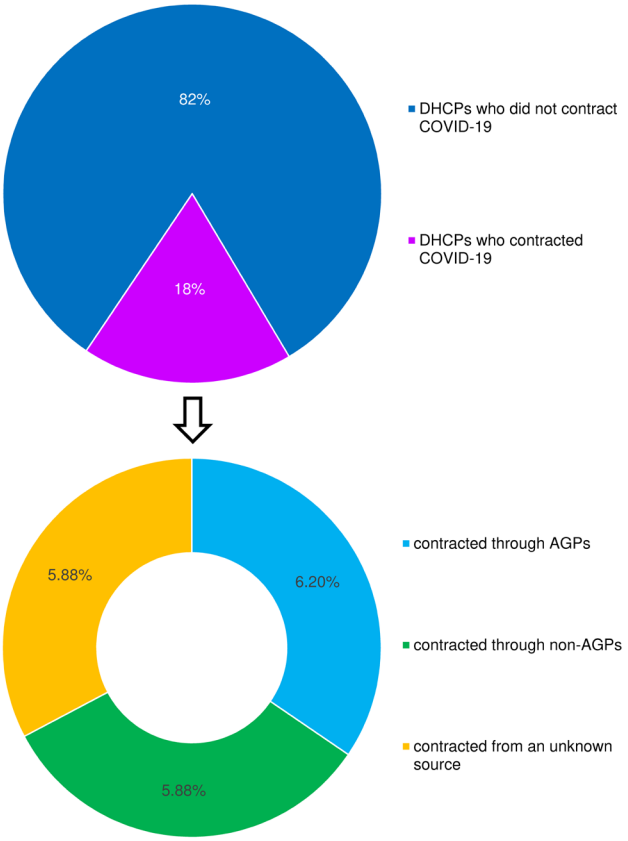

Fig. 1. Percentage of dental healthcare professionals (DHCPs) who contracted coronavirus disease 2019 (COVID-19), together with the source of the infection (N = 629)

Table 2. Number of aerosol-generating procedures (AGPs) performed in a day by all dental healthcare professionals (DHCPs) and the calculation of the infection risk

Independent variable	Number of AGPs performed per day				
	1–3	4–5	6–7	>7	total
Number of DHCPs performing AGPs (n)	339	25	15	250	629
Number of DHCPs who contracted COVID-19 (c)	70	12	6	25	113
Percentage of risk ($c/n \times 100$ [%])	20.65	48.00	40.00	10.00	17.97

According to the obtained data, the PPE used by 39.75% of DHCPs included a surgical mask with a face shield/eye goggles, while 28.78% of DHCPs used an N95 respirator with a face shield/eye goggles. However, 26.70% and 4.77% of DHCPs used surgical masks and N95 respirators, respectively, without a protective face shield/eyewear (Fig. 2).

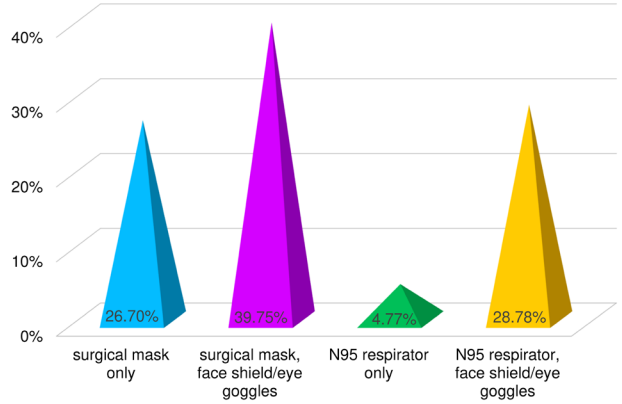

Fig. 2. Types of personal protective equipment (PPE) used by dental healthcare professionals (DHCPs) ($N = 629$)

Figure 3 shows the comparison of the different types of PPE used by the DHCPs who contracted and did not contract COVID-19. The ANOVA test showed significant differences between the types of PPE used with regard to the infection risk ($p < 0.001$). The DHCPs who wore surgical masks with face shields and eye goggles while performing dental procedures had maximum protection, as out of the 250 individuals who used them, only 25 developed COVID-19 (RR (relative risk) = 0.11). Using a surgical mask alone presented an RR of 0.18, using an N95 respirator with a face shield/eye goggles had an RR of 0.25, and using an N95 respirator alone had an RR of 5.00.

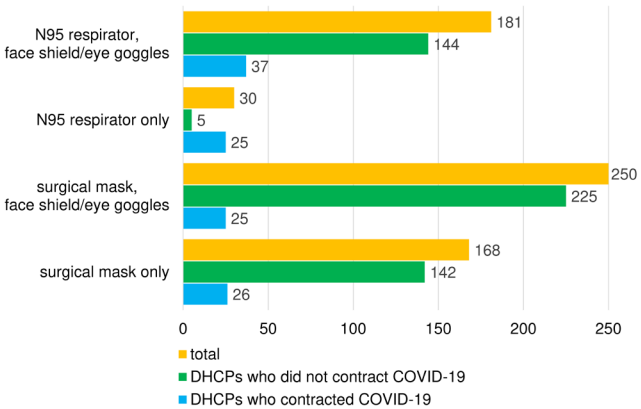

Fig. 3. Comparison of the different types of personal protective equipment (PPE) used by the dental healthcare professionals (DHCPs) who contracted and did not contract coronavirus disease 2019 (COVID-19) ($N = 629$)

Discussion

The Occupational Safety and Health Administration has declared that all AGPs pose a very high risk of COVID-19 transmission from patients with known or suspected infection.[1,21,22] However, the literature still lacks data on the estimation of the risk of COVID-19 spread among dentists and other healthcare professionals (HCPs) with regard to AGPs. Therefore, the aim of this study was to determine the transmission pattern for SARS-CoV-2 through AGPs and non-AGPs among DHCPs. The results of the current study reveal that 113 (17.97%) out of 629 DHCPs contracted COVID-19. Among the 113 DHCPs who contracted COVID-19, 39 (6.20%) reported that they had got infected through AGPs, whereas 37 (5.88%) related the contraction of the disease to non-AGPs, and the remaining 37 (5.88%) were unsure about the source of the infection. Given these results, it can be ascertained that AGPs pose no additional threat to DHCPs as compared to non-AGPs. The results of this study concur with Harding et al.'s review, which states that AGPs do not pose a greater threat of SARS-CoV-2 infection.[23] Wong et al. also reported that in a Hong Kong hospital, 71 HCPs and 49 patients who had come into contact with a SARS-CoV-2-infected patient prior to testing were negative for COVID-19.[24]

The results of the current study also show that the transmission of COVID-19 is not associated with the number of AGPs performed per day, as 61.95% of DHCPs who developed COVID-19 were those who performed 1–3 AGPs in a day. There was a lower number of DHCPs who got infected after performing 4–5, 6–7 or >7 AGPs in a day. In the literature, there is no evidence linking the number of AGPs performed per day with the risk of contracting COVID-19 among DHCPs.

The efficacy of the PPE used by DHCPs was also assessed in the current study. All DHCPs used different types of PPE, depending upon their availability or affordability. The most interesting finding in this study was that, although all face masks prevent the transmission of airborne infections like COVID-19, surgical masks with face shields and eye goggles provided the greatest protection against COVID-19 infection during dental procedures, with an RR of 0.11.

A surgical mask used alone had an RR of 0.18, which was also found to be more efficacious as compared to an N95 respirator with a face shield and eye goggles (RR = 0.25) and an N95 respirator used alone (RR = 5.00). There is no evidence in the literature that coincides with the results of our study and shows a greater efficacy of surgical masks over N95 respirators. However, Johnson et al.[25] and Loeb et al.[26] reported an equal ability of surgical masks and N95 respirators to filter out the influenza virus. Likewise, the latest randomized controlled trial published by Radonovich et al. also demonstrates that there is no significant difference in the efficacy between

surgical masks and N95 respirators.[27] Offeddu et al.[28] and Sommerstein et al.[29] also reported similar results. Therefore, based on the findings of the current study, we advocate the use of surgical masks with face shields or eye goggles during AGPs, since they are cost-effective, convenient, easily obtainable, and safe for the user.

When using N95 masks, individuals may experience increased blood pressure, breathing resistance and heart rate, and diminished exercise performance.[30] These masks are not recommended for people with severe pulmonary or cardiac disease, uncontrolled hypertension, or claustrophobia.[30]

Thus, this study helps to fill the knowledge gaps regarding the transmission routes for SARS-CoV-2, risk to DHCPs and the safety of AGPs. This void in the literature has created fear and uncertainty among DHCPs, which affected their ability to provide dental care when needed.

Conclusions

The illusion that AGPs are high-risk procedures during the COVID-19 pandemic can be set aside to some extent, as this study shows that the chances of contracting COVID-19 during AGPs are the same as in the case of non-AGPs, and that risk severity is not associated with the number of AGPs performed per day. While performing AGPs, a surgical mask with a face shield/eye goggles is the most efficacious PPE against SARS-CoV-2. However, it must be noted that this study used only subjective methods. More definitive conclusions can be drawn through objective methods. Nonetheless, this study will help to implement useful clinical interventions, prevent the wastage of PPE and other resources, and shift the focus to the quality of patient care. In light of these findings, we propose extending dental healthcare services to elective AGPs during the COVID-19 pandemic.

Ethics approval and consent to participate

The study was approved by the institutional Research and Ethics Committee at the Dental College of the Rashid Latif Medical Complex, Lahore, Pakistan (No. of approval: RLDC/006080/20). Written informed consent was obtained from all participants.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Effect of oral antiseptics on the viral load of SARS-CoV-2: A randomized controlled trial

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Abstract

Background. In the oral cavity, which plays an important role in the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), it is possible to reduce the viral load of SARS-CoV-2 with antiseptics, thereby minimizing the transmission of the virus during dental procedures.

Objectives. The aim of this study was to clinically evaluate the effect of the hypochlorous acid (HClO) and povidone-iodine (PVP-I) solutions on the oral viral load of SARS-CoV-2.

Material and methods. This randomized controlled trial was conducted on 75 patients hospitalized in the COVID-19 ward of a local hospital. All the patients included in the study were within the first 24 h of hospitalization and the first 5 days of coronavirus disease 2019 (COVID-19) symptoms. The viral load of mouthwash samples was measured with the cycle threshold (*Ct*) value of SARS-CoV-2 through a real-time reverse transcription polymerase chain reaction (RT-PCR). The patients were divided into 3 groups. The effect on the patient's SARS-CoV-2 viral load was investigated after gargling the mouths and throats for 30 s with HClO, PVP-I and isotonic saline. First, a sample was taken after gargling with isotonic saline, then another sample was taken after gargling for 30 s with a particular antiseptic to determine the viral load of SARS-CoV-2.

Results. Comparing the before and after mouthwash samples from all 3 groups, there were no statistically significant differences in the *Ct* values before and after gargling ($p > 0.05$). However, there were statistically significant differences in the number of negative samples after the use of HClO and PVP-I, which were positive before gargling ($p < 0.05$).

Conclusions. In the light of the data obtained in this study, there is insufficient evidence that gargling with HClO or PVP-I reduces viral load. Taken together, these findings imply no role for antiseptics in the transmission of SARS-CoV-2 by the aerosol generated during dental procedures, or more generally, SARS-CoV-2 infection control.

Keywords: viral load, COVID-19, SARS-CoV-2, hypochlorous acid, povidone-iodine

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Introduction

On March 11, 2020, the World Health Organization (WHO) declared that the coronavirus disease 2019 (COVID-19) outbreak was a pandemic phenomenon. As of March 21, 2022, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for COVID-19, has infected more than 470 million people worldwide, with nearly 6 million deaths. COVID-19 has been reported to have potentially harmful effects on people's physical and mental health, causing economic uncertainty and social isolation as well as deaths.[1,2] The WHO classified SARS-CoV-2 as an airborne pathogen and reported that it was transmitted by close contact with the aerosols and droplets emitted by asymptomatic and pre-symptomatic individuals as well as those with symptoms.[3] Epithelial cells in the oral mucosa and salivary glands contain a large number of viral entrance factors for SARS-CoV-2, such as angiotensin-converting enzyme 2 (ACE2) and the transmembrane protease serine subfamily (TMPRSS) enzymes.[4] Mild to moderate cases of COVID-19 infection have proven to be associated with oral symptoms,[5] and therefore patients with infectious diseases may present with oral problems.[6] The contamination of aerosol from the oral cavities of patients is a potential danger to dentists, assistant staff and other patients,[7] and thus the World Economic Forum has recognized dentists as one of the professions with the highest risk for COVID-19.[8] Therefore, in addition to strict protection measures, saliva is thought to be important in preventing the transmission of COVID-19, especially during oral treatment, in terms of reducing viral load in COVID-19 patients.[9]

Antiseptics can be used effectively to reduce viral load, lowering the risk of respiratory tract infections.[10] It has been reported that oral antiseptics may be beneficial both in reducing the severity of the disease by lessening the pathogenicity of the virus, and also in preventing the virus from remaining in the mouth and being transferred from the body to the outside.[11] An important step in reducing viral load is the use of antiseptics with an ingredient that exhibits antiviral effects, such as hydrogen peroxide (H_2O_2), povidone-iodine (PVP-I), chlorhexidine (CHX), cetylpyridinium chloride (CPC), cyclodextrins, Citrox®, phthalocyanine, essential oils, or hypochlorous acid (HClO). Reviews of the available research concluded that antiseptics with antiviral effects could decrease the severity of COVID-19 by reducing the SARS-CoV-2 oral viral load, lowering the risk of transmission, and therefore might be useful in the current pandemic situation.

One of the antiseptics approved for the fight against SARS-CoV-2 is HClO.[12] It has a broad spectrum, works quickly and is considered to be very safe. Currently, it is used to control and prevent a variety of skin and mucosal infections.[13] Hypochlorous acid destroys viruses through chlorination by forming chloramines and nitrogen-centered radicals, causing single- and double-stranded DNA breaks, rendering nucleic acid inoperative, and inactivating the virus.[14] Presently, 0.01% HClO is approved by the Australian Register of Therapeutic Products (ARTG) as an effective disinfectant against COVID-19.[15]

Povidone-iodine has been shown to be an effective antiseptic against enveloped and non-enveloped viruses; its antimicrobial activity has been used for years.[16] An in-vitro study conducted in Japan found that the applied PVP-I mouthwash showed antiviral activity against several different viruses, such as adenovirus, rotavirus, poliovirus (types 1 and 3), coxsackievirus, rhinovirus, herpes simplex virus (HSV), rubella virus, measles virus, mumps virus, influenza virus, and human immunodeficiency virus (HIV).[17] In another in vitro study, it was reported that 0.23% PVP-I rapidly inactivated severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), influenza A virus subtype H1N1, and rotaviruses with a 15-second action time.[18]

Nasopharyngeal swabs are considered the gold standard as the diagnostic biomaterials recommended for suspected cases of SARS-CoV-2 infection. However, it has been reported that the collection of such specimens is not suitable for all patient types.[19] As an alternative to nasopharyngeal swabs, saliva has been reported to be a safe and reliable tool for the diagnosis of COVID-19, offering greater safety, and logistic and economic benefits.[20]

Due to the lack of clinical data, the aim of this study was to clinically evaluate the effect of the HClO and PVP-I solutions on the viral load of SARS-CoV-2 in COVID-19 patients. The null hypothesis was that there would be no difference in reducing the SARS-CoV-2 viral load between the HClO or PVP-I solutions.

Material and methods

Study design

The present study is a randomized, blind, controlled clinical trial investigating the effect of oral antiseptics with the HClO and PVP-I solutions on the intraoral viral load of SARS-CoV-2-positive patients hospitalized in the isolation ward of Erzurum Regional Training and Research Hospital, Turkey. This study was approved by the Institutional Review and Ethics Board of Erzurum Regional Training and Research Hospital (approval No. 2021/16-237), conducted according to the 2008 Declaration of Helsinki with later amendments and registered at ClinicalTrials.gov (NCT 05214196). The written informed consent was obtained from all participants. The randomization of the groups was performed using a computer program.

Power analysis

The sample size was determined by $\alpha = 0.05$ and a power (1-β) of 80%. Due to the variability value (σ), a 0.37 change in viral load was used. Relying on this data, the minimum number of patients required for this study was computed as 75, for 3 groups.

Patients and samples

A flow chart of the study is presented in Fig. 1. A total of 75 adult patients who had the presence of SARS-CoV-2 RNA confirmed by real-time reverse transcription polymerase chain reaction (RT-PCR), had COVID-19 infection and were able to follow hospital treatment guidelines were included in the study. According to the inclusion criteria, all patients had a high viral load (cycle threshold (*Ct*) value <25) in the nasopharyngeal samples on admission to hospital and were within the first 5 days of COVID-19 symptoms. Samples were collected within the first 48 h after hospital admission (2 ±1 days after the SARS-CoV-2 RT-PCR test). Individuals, irrespective of gender, were at least 20 years of age and at most 83 years of age. In addition, it was necessary to have the mental ability to understand the instructions given and not to have any physical disability to implement them. The exclusion criteria were severe acute or chronic medical or psychiatric condition, a history of significant adverse effects following the use of oral hygiene products, such as a toothpaste and antiseptics, active uncontrolled thyroid disease, developmental/cognitive disability, pregnancy, and presently undergoing the radioactive iodine therapy. Those who were intubated and supported with a mechanical respirator were excluded from the study. Before brushing their teeth and eating/drinking anything in the early morning, the participants were asked to open 5-milliliter vials of sterile 0.9% saline (Gifrer®; Haks® Group, Istanbul, Turkey) and empty the contents into their mouths. The sample was gargled, and then collected into a sterile container. Next, 20 mL of 0.02% HClO solution was given to 25 patients, 20 mL of 0.5% PVP-I solution was given to 25 patients and 20 mL of 0.9% saline solution was given to 25 patients. Mouth and throat gargling was performed with the solutions for 30 s. It was insured that patients did not eat or drink anything for 30 min; then, they gargled with 5 mL of 0.9% saline for 30 s, similar to the 1st sample, and this sample was then collected into a sterile container. All samples were cryopreserved at –80°C until the time of analysis. The patients' demographic and clinical data was obtained from hospital electronic records.

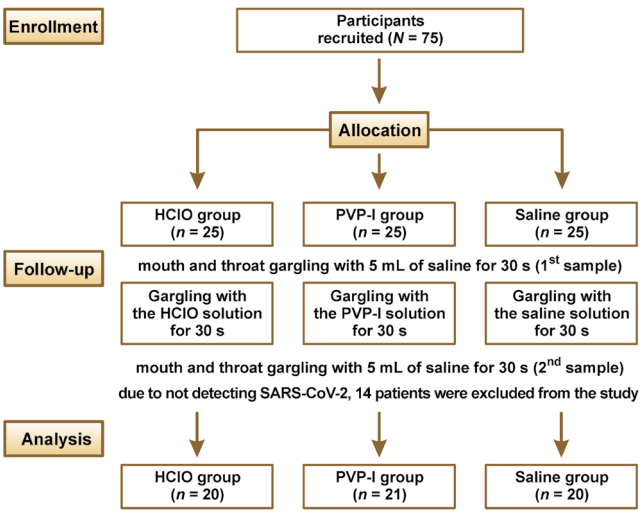

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram
HClO – hypochlorous acid; PVP-I – povidone-iodine; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2.

Detection of viral load

The effectiveness of antiseptics of the HClO, PVP-I and isotonic saline solutions in the initial and final samples taken from each patient was investigated by means of the real-time RT-PCR method. The initial diagnosis of patients with the nasopharyngeal samples and the investigation of the presence of SARS-CoV-2 nucleic acid in the mouthwash samples were performed with the use of the Bio-Speedy® SARS-CoV-2 Double Gene RT-qPCR kit (Bioeksen R&D Technologies, Istanbul, Turkey) and the Rotor-Gene Q 5plex real-time PCR instrument (Qiagen, Hilden, Germany). This kit is a real-time, one-step RT-PCR test that targets the *ORF1ab* and *N* genes in the viral genome, including the extraction and sample quality control targeting the human RNase P gene. Both positive and negative controls were included in each run to generate a valid result. All of the analyses were carried out in the COVID-19 reference laboratory by the staff experienced in molecular methods. The Bio-Speedy SARS-CoV-2 Double Gene RT-qPCR test has been approved by the U.S. Food and Drug Administration (FDA) and added to the Emergency Use Authorization (EUA) list; it provides results with 99.6% sensitivity and 100.0% specificity according to the manufacturer's package insert. Since the RT-PCR tests used in the detection of SARS-CoV-2 are qualitatively designed, viral load was measured through the surrogate markers of the *Ct* values for SARS-CoV-2-specific gene targets, using the RT-PCR assays applied to the specimens. The RT-PCR result was considered negative when the *Ct* value could not be measured up to 40 cycles. Although the *Ct* values do not provide a complete quantitation, they are semi-quantitative in the measurement of viral load.

Statistical analysis

The results were described as mean ± standard deviation ($M \pm SD$). The normal distribution suitability of the parameters was determined with the Kolmogorov–Smirnov and Shapiro–Wilk tests. Since the baseline and post-gargle values showed normal distribution, the paired-samples *t* test for dependent samples was used to compare the means of numerical data from 3 dependent groups. The one-way analysis of variance (ANOVA) was performed to examine differences

between the groups and the independent samples *t* test was used to examine differences between the paired groups. The Wilcoxon test was used for the analysis of the positive and negative results of the SARS-CoV-2 test, which did not show normal distribution, and the Kruskal–Wallis test was used for comparisons between the groups. A *p*-value <0.05 was considered to be statistically significant. The statistical analysis was performed with the use of the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA).

Results

The presence of SARS-CoV-2 in the patients of the present study was confirmed through the initial gargle sample. Fourteen of 75 patients were excluded from the study, as SARS-CoV-2 could not be detected in their samples. The age and gender distribution of the 61 patients included is provided in Table 1. Table 1 also shows the distribution of the positive and negative results of the SARS-CoV-2 test. A *Ct* value ≥40 was considered negative. In the comparisons made within the groups, it was determined that the HClO and PVP-I groups showed statistically significant negative results ($p < 0.05$) (Fig. 2). However, in the comparison between the groups, it was found that there were no significant differences between the groups ($p > 0.05$).

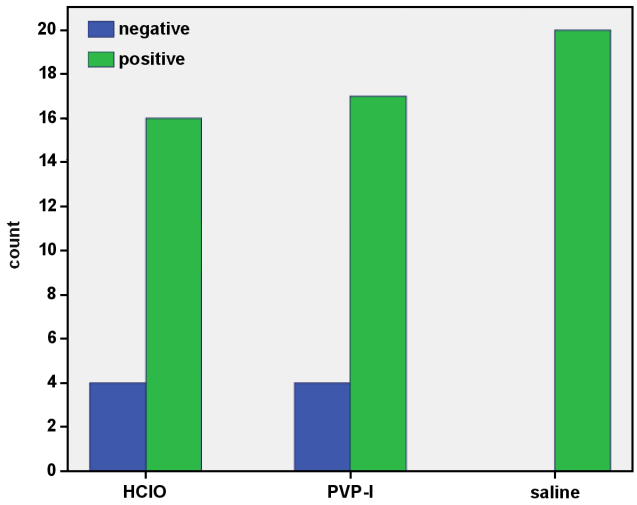

Fig. 2. Number of negative and positive samples as determined by real-time reverse transcription polymerase chain reaction (RT-PCR) post-gargle by groups ($p < 0.05$)

Table 1. Distribution of age, gender and the cycle threshold (*Ct*) values at baseline and post-gargle

Patient No.	HClO group (*n* = 20)					PVP-I group (*n* = 21)					Saline group (*n* = 20)				
	age [years]	gender	B	PG	PG result	age [years]	gender	B	PG	PG result	age [years]	gender	B	PG	PG result
1	47	F	17.24	18.70	+	43	F	32.20	25.48	+	20	M	24.53	25.43	+
2	20	F	29.82	23.99	+	58	M	24.55	40.00	–	75	F	25.76	25.30	+
3	47	M	29.41	27.82	+	78	F	29.90	28.50	+	79	F	33.17	34.09	+
4	52	F	24.25	19.59	+	30	M	30.65	31.09	+	61	F	22.64	22.66	+
5	72	F	21.90	21.15	+	35	F	23.83	23.37	+	74	F	24.13	25.32	+
6	33	F	26.76	27.88	+	50	M	33.15	40.00	–	83	F	28.54	27.68	+
7	40	M	29.38	23.77	+	51	M	24.12	25.33	+	87	M	21.70	25.62	+
8	60	M	30.39	30.31	+	45	M	29.02	40.00	–	53	F	29.80	31.75	+
9	40	M	24.30	23.10	+	32	M	30.18	40.00	–	35	M	32.06	35.92	+
10	56	M	28.70	40.00	–	35	M	21.84	20.76	+	57	F	22.58	20.38	+
11	40	F	22.90	27.61	+	33	F	26.30	27.00	+	61	M	26.67	27.37	+
12	33	M	37.50	40.00	–	20	M	20.63	24.14	+	33	F	28.54	27.68	+
13	65	M	30.47	33.56	+	68	F	27.78	26.96	+	65	M	20.86	24.82	+
14	70	F	33.36	40.00	–	77	F	28.97	28.71	+	70	M	27.85	30.75	+
15	81	F	32.94	31.05	+	54	M	27.29	26.63	+	67	M	23.12	21.63	+
16	48	F	29.52	28.27	+	55	M	30.32	32.30	+	48	M	27.29	26.63	+
17	68	F	35.43	35.43	+	66	F	27.01	25.57	+	20	F	22.48	20.99	+
18	30	M	34.17	40.00	–	59	F	23.12	21.63	+	30	M	25.66	26.89	+
19	20	F	22.34	19.38	+	68	F	30.72	30.82	+	20	M	31.13	31.88	+
20	83	F	31.64	30.57	+	40	M	32.22	31.96	+	45	F	25.78	26.68	+
21			NA			44	F	29.03	31.34	+			NA		
M ±*SD*	50.57 ±18.39		28.62 ±5.15	29.11 ±7.24		49.57 ±16.08		27.75 ±3.58	29.60 ±6.08		54.28 ±21.23		26.29 ±2.63	26.30 ±4.64	
p-value	–	–	–	–	<0.05*	–	–	–	–	<0.05*	–	–	–	–	>0.05

M – mean; *SD* – standard deviation; B – *Ct* value at baseline; PG – *Ct* value post-gargle; M – male; F – female; + SARS-CoV-2-positive; – SARS-CoV-2-negative; NA – not applicable; * statistically significant (Kruskal–Wallis test).

Table 2 shows the distribution of the SARS-CoV-2 RT-PCR *Ct* values among the groups which gargled with different solutions. Although there was an increase in the mean *Ct* values of the patients who gargled with PVP-I, no statistically significant differences were found between the groups before and after gargling ($p > 0.05$). It was also observed that there were no statistically significant differences in the values within the groups ($p > 0.05$) (Fig. 3,4).

Table 2. Comparison of the mean cycle threshold (*Ct*) values among the groups

Time point	*Ct* values			*p*-value†
	HClO group ($n = 20$)	PVP-I group ($n = 21$)	saline group ($n = 20$)	
Baseline	28.62 ±5.15	27.75 ±3.58	26.29 ±3.63	>0.05
Post-gargle	29.11 ±7.24	29.60 ±6.08	26.30 ±4.64	>0.05
p-value‡	>0.05	>0.05	>0.05	–

Data presented as *M* ±*SD*. † one-way ANOVA test; ‡ paired-samples *t* test.

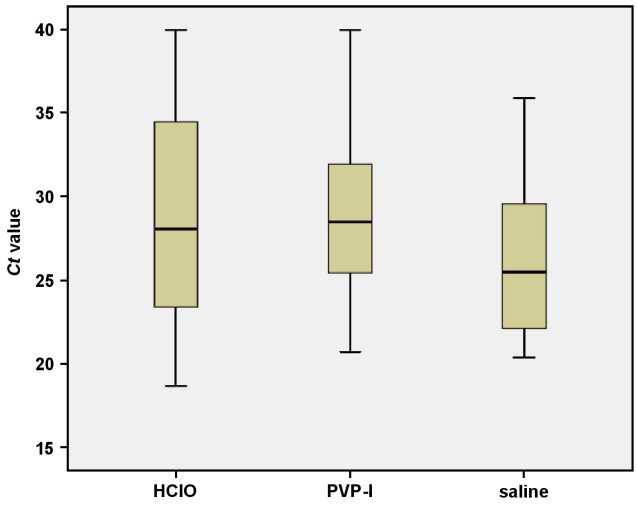

Fig. 3. Cycle threshold (*Ct*) values of the groups at baseline as determined by real-time RT-PCR ($p > 0.05$)

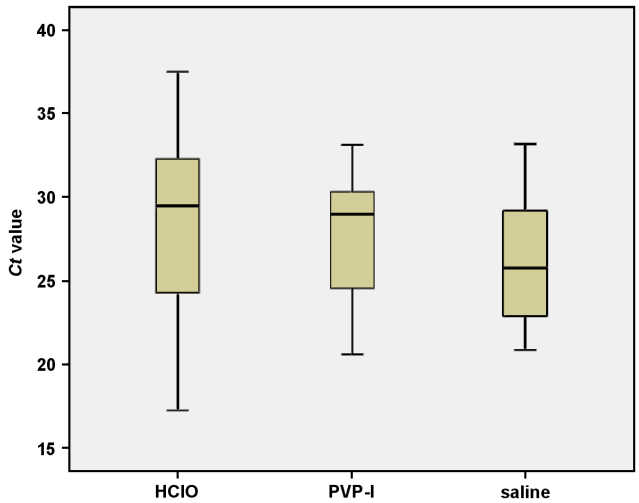

Fig. 4. Cycle threshold (*Ct*) values of the groups post-gargle as determined by real-time RT-PCR ($p > 0.05$)

No adverse clinical events related to the antiseptics were reported (burning in the mouth, allergy, taste disorder, or a dry mouth).

Discussion

The aim of this study was to investigate and compare the effectiveness in reducing the SARS-CoV-2 oral viral load of two different solutions of HClO and PVP-I with isotonic saline. The data from this clinical study is consistent with the null hypothesis: there was no statistically significant difference in reducing the SARS-CoV-2 viral load in either the HClO group or the PVP-I group as compared to the saline group. However, there were statistically significant differences in the number of patients who were SARS-CoV-2-negative after gargling in both groups as compared to the control group.

A recent study reported that the application of oral antiseptics in the oropharyngeal region, one of the viral replication centers in the early asymptomatic stages of COVID-19, had the potential to reduce viral load in the oropharynx.[21] It is known that the envelope of SARS-CoV-2 is highly sensitive to chemical agents that disrupt lipid biomembranes.[22] Therefore, chemical antisepsis is important to decontaminate the open surfaces of the body, such as the mouth. Povidone-iodine has often been suggested for usage as an antiseptic before dental procedures.[23–28] However, in a clinical study conducted by Seneviratne et al., it was found that there was no significant difference in the *Ct* values in the COVID-19 patient group using PVP-I as compared to control.[9] In this respect, it is compatible with our study. Unlike our study, Seneviratne et al. repeated sampling at different time intervals, and also investigated fold changes. The fold change of the PVP-I group was significantly increased at 6 h, whereas no change was observed in the control group.[9] In another clinical study, Chaudhary et al. reported that there was no statistically significant decrease in viral load at both 15 and 45 min in the PVP-I group as compared to the isotonic saline group.[29] These findings are also consistent with the present study. The results of only one of the comprehensive clinical studies investigating viral load in COVID-19 patients were not compatible with our research.[24] Both the sampling technique and time could be counted as the reasons for the disagreement. Like other viruses, SARS-CoV-2 replicates in living cells. Although the applied antiseptics could affect the viral particles outside the cell, they might have not been able to affect the viral particles inside the cell. Therefore, it is thought that antiseptics are effective in the first stage of contamination, before they enter the cell in the early period. This could be the reason why we did not detect any differences in the SARS-CoV-2 RT-PCR *Ct* values before and after the antiseptic application, since this study was conducted on hospitalized patients, i.e., advanced patients. However, it should be noted that RT-PCR positivity cannot be considered as the evidence of an infective virus.

To the best of our knowledge, there has been no published clinical study on the oral antiseptic HClO against SARS-CoV-2 so far. This is the first study to clinically investigate HClO as an oral antiseptic against the SARS-CoV-2 viral load, adding new information to the literature. To date, there are only in vitro studies evaluating the virucidal effect of HClO on SARS-CoV-2.[30–32]

SARS-CoV-2 has been detected in asymptomatic patients with the viral load reported to be similar to that in symptomatic patients.[33] Since patients with no or minimal symptoms often report for dental treatment, they are a danger to clinicians just as much as individuals with symptoms. In addition, hospitalized patients were chosen for the study group because of a high viral load due to an increase in the severity of the disease. It was preferred to collect SARS-CoV-2 samples from the patients in the early morning on the 1st day of hospitalization. Studies have reported that the viral load studied in samples is the highest at 4–8 days, when symptoms appear.[34,35] Furthermore, some authors have suggested the use of the throat gargle sampling method, which shows a higher viral load than a nasopharyngeal swab and an oropharyngeal swab.[36,37] When this sampling technique is used, it is easily tolerated by the patient, not affected by anatomy and less dependent on the specially trained people to collect specimens. When the patient is given written or verbal instructions, they can apply the instructions on their own. There is a minimal risk of contamination, as the method does not require close contact. In addition, it has been reported that the saliva collection method had a sensitivity of 79%, while the saline mouth and throat gargle method had a sensitivity of 98%.[38]

Limitations

This study has several limitations. SARS-CoV-2 was detected in only 61 of the 75 samples obtained. The reason for negative results is the sensitivity of saliva samples. In a study using the same kit as ours, SARS-CoV-2 RNA positivity in the saliva samples of COVID-19 patients was found at the level of 60%,[39] and it was 70% in another study performed with a different kit.[40] The fact that 80% of the samples were positive in our study shows that the sample quality was good. Both positive and negative controls were included in each run to generate a valid result. All of the analyses were carried out in the COVID-19 reference laboratory by the staff experienced in molecular methods. The Bio-Speedy SARS-CoV-2 Double Gene RT-qPCR test has been approved by the U.S. Food and Drug Administration (FDA) and added to the Emergency Use Authorization (EUA) list; it provides results with 99.6% sensitivity and 100.0% specificity according to the manufacturer's package insert. The applied standards limited the number of samples acquired for the research. Furthermore, since the RT-PCR *Ct* value was used for the detection of the SARS-CoV-2 viral load (together with live and non-infective viral particles), the viability of the virus could not be evaluated. It would be useful to culture SARS-CoV-2 in a cell culture for active virus replication; however, this requires special laboratory conditions, such as biosafety level 4. For that reason, we conducted a preliminary study that used a semi-quantitative and feasible method to measure viral load in the samples. Another limitation of the study is that it was conducted on symptomatic hospitalized patients. When a similar analysis is performed on asymptomatic patients, different results might be obtained.

Conclusions

In line with the obtained results, it was determined that gargling with oral antiseptics for 30 s is not sufficient to reduce the viral load of SARS-CoV-2. Gargling with an oral antiseptic before dental procedures can provide a false sense of security, so proper personal protective equipment (PPE) should always be worn before any aerosol-generating procedures in the dental clinic, and four-handed dentistry, the use of a rubber dam or high evacuation suction are advisable to minimize droplet splashing.

Trial registration

This study was registered at ClinicalTrials.gov (NCT 05214196).

Ethics approval and consent to participate

This study was approved by the Institutional Review and Ethics Board of Erzurum Regional Training and Research Hospital (approval No. 2021/16-237) and conducted according to the 2008 Declaration of Helsinki with later amendments. The written informed consent was obtained from all participants.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Assessment of attitudes and practices regarding oral healthcare during the COVID-19 pandemic among the parents of children aged 4–7 years

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Abstract

Background. The coronavirus disease 2019 (COVID-19) pandemic has brought about radical changes in our habits and lifestyles. The suspension of schools has led children to spend long hours at home, with reduced socialization, and changes in dietary patterns, oral hygiene practices and sleep routines. During a pandemic, appropriate oral health management and disease prevention are very important for the child's oral and general health.

Objectives. The aim of this study was to assess the attitudes and practices of parents with regard to their children's oral healthcare, dietary habits and dental care during the COVID-19 pandemic.

Material and methods. This cross-sectional study included 381 Indian parents of children aged 4–7 years. A self-instructed questionnaire was designed in English using the Google Forms platform. The questionnaire consisted of 4 parts: sociodemographic data; dietary habits of the child; oral hygiene measures; and dental information. The collected data was analyzed using descriptive and analytical statistics (the χ^2 test).

Results. Among the children included in the study, 48% of those who experienced dental problems during the pandemic consumed more snacks and packaged foods between meals. Among the parents, 80% reported that their children used electronic devices at mealtimes, and 60% reported the food pouching habit in their children. A total of 71% of parents assisted their child at tooth brushing, while only 28% of the parents would take their child to the dental clinic for treatment.

Conclusions. This study highlights the shortfalls in attitudes and practices among parents in relation to dietary habits, oral hygiene measures and the use of dental services during the COVID-19 pandemic regarding their children. This could be attributed to a lack of awareness, the fear of exposure and the inconveniences faced by parents.

Keywords: teledentistry, COVID-19, pediatric dentistry, oral hygiene measures, dietary changes

Introduction

The World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) a public health emergency of international concern (PHEIC) on January 30, 2020, and characterized the outbreak as a global pandemic on March 11, 2020.[1] As of October 2021, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had spread across the globe and infected over 236 million people.[2] The SARS-CoV-2 is regarded as a highly contagious virus that spreads mainly through the small droplets expelled from the nose or the mouth when an infected person speaks, coughs or sneezes.[3] Clinical symptoms typically include a dry cough accompanied by fever.[4] Difficulty in breathing and fatigue, along with other, less typical symptoms also occur.[5] According to a study on more than 2,000 child patients with suspected or confirmed COVID-19, over 90% were asymptomatic or presented mild to moderate symptoms.[6] Of the 13.5 million cases reported in those under 20 years of age, 31% were among children aged 0–9 years.[7] In India, 11.9% of the reported COVID-19 infections are among children and adolescents.[8] The highly transmissible Omicron variant has affected children more than previous variants, but its symptoms are significantly milder.[9]

Since there is no definitive treatment protocol in place, different countries announced nationwide lockdowns to control the spread of the disease.[10] Sudden and radical changes occurred in habits and lifestyles, with a drastic reduction in all forms of socialization.[11] The suspension of schools and sports activities led children to spend long hours at home, which resulted in disturbances in dietary patterns, oral hygiene practices and sleep routines in children.[3] However, certain countries, like Sweden, avoided a complete nationwide lockdown.

Concerns regarding the safety of dentists and children led to a reduction in routine dental visits, compromising preventive appointments. This was further compounded by the unavailability of pediatric dental services in many places. Parents contacted dentists only for emergencies, such as acute pulpitis, dental trauma, and oral and maxillofacial infections that caused swelling.[10] Many resorted to a medical prescription and postponed dental treatment.

A survey conducted in Russia in 2020 collected data from 166 pediatric patients aged 1–17 years. Acute pain in primary molars in children aged 4–7 years was the most prevalent finding (62%).[12] Similar scenarios have been observed by pediatric dentists in India, although no documentation has been reported to date.

During the pandemic, appropriate oral health management and disease prevention are necessary for the general health of the child. Parents play a key role in implementing measures to foster better habits.[13] There is a directly proportional relationship between parental attitudes and the health of children.[10] Thus, this study was conducted to assess attitudes and practices regarding children's oral healthcare during the COVID-19 pandemic among parents with children aged 4–7 years.

Material and methods

Study duration and the ethics approval

A cross-sectional study was carried out from August 1 to October 1, 2021, during the phased opening stage. Approval was obtained from the Ethics Committee at the School of Dentistry of D.Y. Patil Deemed to be University, Navi Mumbai, India (IREB/2021/PEDO/21).

Data collection

The convenience sampling technique was employed for this study. The questionnaire was made available on the Google Forms platform (Google, Mountain View, USA). The link to the questionnaire was sent through the WhatsApp application (Meta Platforms, Inc., Menlo Park, USA) to the parents of schoolchildren, and to the parents who arrived for a dental visit at the Department of Pedodontics and Preventive Dentistry of the D.Y. Patil Deemed to be University School of Dentistry. The sample size was calculated by considering the representative sample size, using a 95% confidence level and a margin of error of 5%. The sample size was then estimated at a minimum of 375 respondents. The nature of the study was explained in brief and the anonymity of the respondents was affirmed. The parents were also informed that the completion of the questionnaire would be considered as consent to participate. A total of 381 parents agreed to participate in the study. The inclusion criteria were specified as all parents of children between 4 and 7 years of age. Parents who did not complete the questionnaire were excluded from the study.

This study followed the established guidelines for reporting medical surveys.[14] The questionnaire was designed by adapting questions from previous surveys in English. A pilot study was conducted among 10 parents to check the validity and reliability of the questionnaire. After necessary modifications to a few questions, the implementation phase began.

The revised questionnaire consisted of 4 parts: sociodemographic data; dietary habits of the child (7 questions); oral hygiene measures (3 questions); and dental information (6 questions) (Table 1). Multiple choices were provided for each question and the participants had to mark the option they considered appropriate.

Statistical analysis

The data was statistically analyzed using GraphPad Prism, v. 8.4.3 (GraphPad Software, Inc., San Diego, USA). Descriptive analysis was performed for the demographic data. The χ^2 test was used to test for statistically significant differences between the variables in the survey. Statistical significance was established at $p \leq 0.05$.

Table 1. Questionnaire

Parameters	Questions
Sociodemographic data	age/gender of the parent
	age/gender of the child
	socioeconomic status (education/occupation/income)
Dietary habits of the child	Was there any change in the amount of food consumed by your child during the pandemic?
	Was there any change in the eating habits of your child during the pandemic?
	Was there an increase in snacking between meals by your child?
	What was the frequency of consumption of cariogenic food (sweets/chocolate/cookies) during the pandemic?
	Does your child use electronic devices (tablet/phone/TV/computer) while eating or snacking?
	Does your child tend to pouch food in the mouth during meals, which leads to an extended mealtime?
	Has there been a change in the sleep cycle of your child since the pandemic began?
Oral hygiene measures	How often does your child brush his/her teeth?
	What measures did you take to maintain your child's oral health during the pandemic?
	Were you able to assist your child at tooth brushing during the pandemic?
Dental information	Did you take your child to a dentist before the pandemic?
	Did your child experience any toothache/cavity/swelling during the pandemic?
	Did your child experience any dental trauma during the pandemic?
	Would you take your child to a dental clinic during the pandemic?
	Do you think your child could get infected with COVID-19 during dental treatment?
	What would be your mode of contact with the dentist, if required?

COVID-19 – coronavirus disease 2019.

Results

The sociodemographic details of the participating parents are presented in Table 2 and Fig. 1. Classifying the parents according to the modified Kuppuswamy scale,[15] the majority of them were in the upper class (40.7%) and upper middle class (30.4%) categories. Based on the total number of responses (381), 138 (36%) children experienced dental problems during the pandemic.

Dietary factors

Among the children who experienced dental problems during the pandemic, 45 (33%) showed an increase in the amount of food consumed during the pandemic. However, the parents of 80 (58%) children reported no change in food consumption. Differences between the groups proved to be statistically significant ($p < 0.01$) (Fig. 2). Fifty-nine (43%) children who experienced dental problems during the pandemic consumed more processed foods, such as sweets and

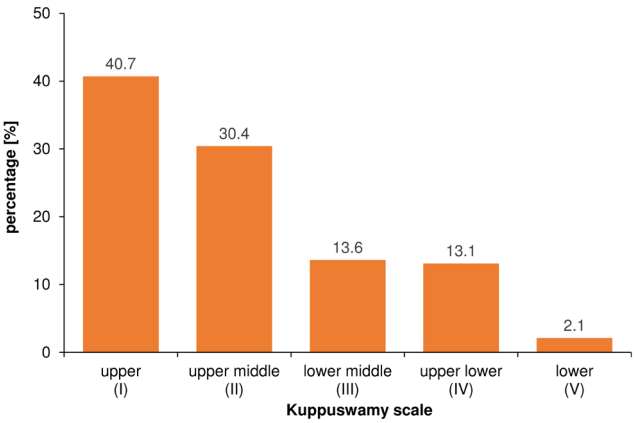

Fig. 1. Socioeconomic status of the parents according to the modified Kuppuswamy scale

Table 2. Characteristics of the study population ($N = 381$)

Characteristics		*n* (%)
Gender of the child	boys	206 (54.1)
	girls	175 (45.9)
Parents' education	illiterate	1 (0.3)
	diploma	61 (16.0)
	primary school	7 (1.8)
	middle school	35 (9.9)
	high school	53 (13.9)
	professional degree	224 (58.8)
Parents' occupation	unemployed	57 (15.0)
	elementary occupation	16 (4.2)
	craft and related trades workers	4 (1.0)
	plant and machine operators and assemblers	3 (0.8)
	skilled agricultural and fishery workers	13 (3.4)
	service workers and shop and market sales workers	34 (8.9)
	technicians and associate professionals	23 (6.0)
	legislators, senior officials and managers	54 (14.2)
	professionals	177 (46.5)

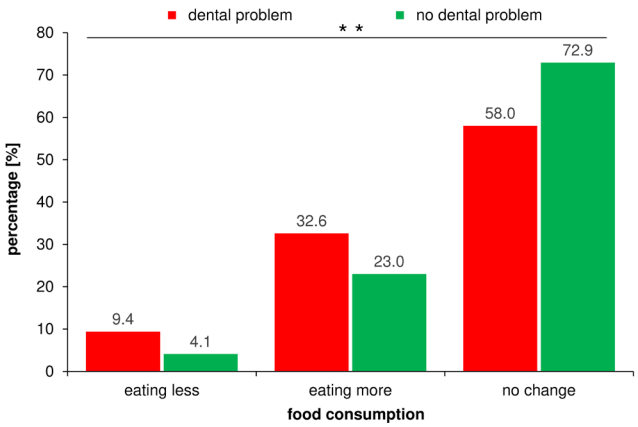

Fig. 2. Relationship between dental problems and changes in food consumption

** statistically significant ($p < 0.01$).

cookies, and soft drinks, with merely 17 (12%) children having consumed more fresh fruits and vegetables during their time away from school. As shown in Fig. 3, differences in the percentages of children in the context of their eating habits were also found to be statistically significant ($p < 0.0001$). Additionally, it was determined that 66 (48%) children who experienced dental problems during the pandemic consumed more snacks and packaged foods between meals. There were statistically significant differences between the groups ($p = 0.0003$) (Fig. 4). On average, children who consumed snacks once a day (31%) and several times a day (29%) experienced more dental issues ($p < 0.0001$) (Fig. 5).

Among the 381 respondents, 304 (80%) parents reported that their children used electronic devices at mealtimes and while snacking between meals. Hence, out of the 138 children who experienced dental problems,123 (89%) accounted for those who used electronic devices ($p < 0.001$) (Fig. 6). Another significant finding was the habit of food pouching during meals in 82 (60%) of the children who experienced dental problems ($p < 0.0001$) (Fig. 7).

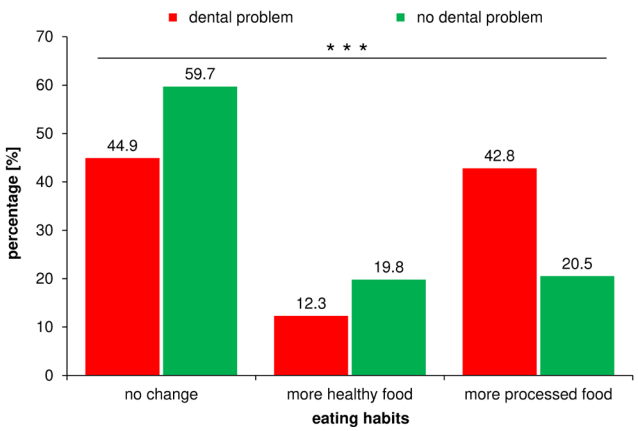

Fig. 3. Relationship between dental problems and changes in eating habits

*** statistically significant ($p < 0.001$).

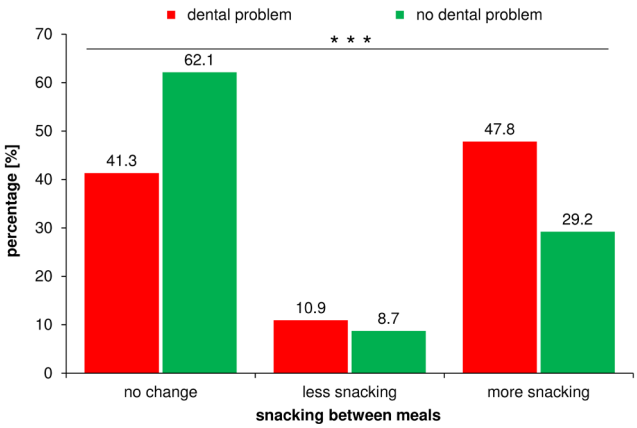

Fig. 4. Relationship between dental problems and snacking between meals

*** statistically significant ($p < 0.001$).

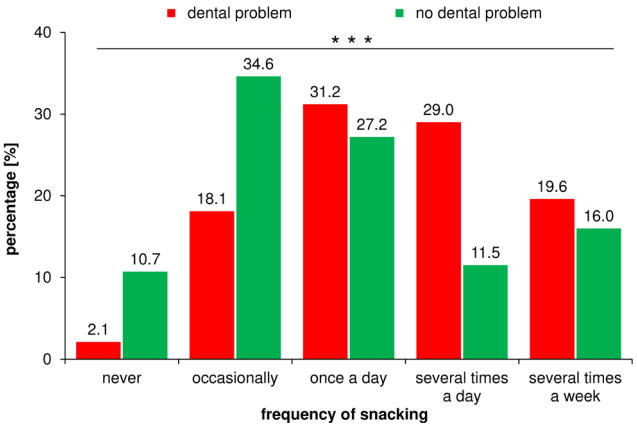

Fig. 5. Relationship between dental problems and the frequency of snacking

*** statistically significant ($p < 0.001$).

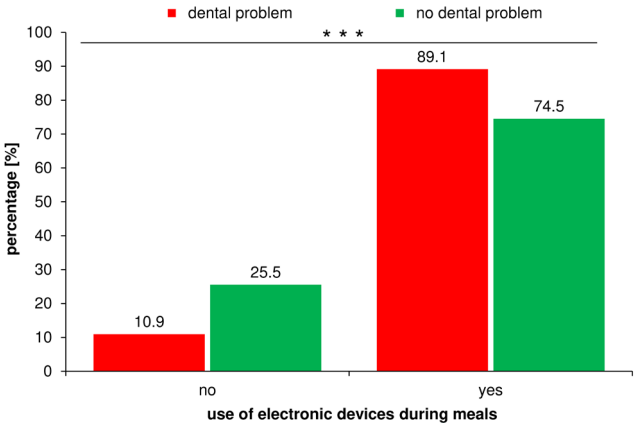

Fig. 6. Relationship between dental problems and the use of electronic devices during meals

*** statistically significant ($p < 0.001$).

Oral hygiene factors

Most parents reported that the frequency of tooth brushing by their children did not change when they were not going to school. Eighty-nine (65%) children who experienced dental problems during the pandemic brushed their teeth once daily ($p = 0.012$) (Fig. 8).

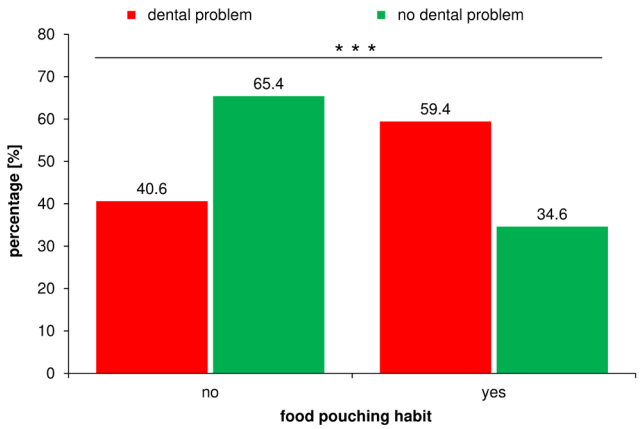

Fig. 7. Relationship between dental problems and the food pouching habit
*** statistically significant ($p < 0.001$).

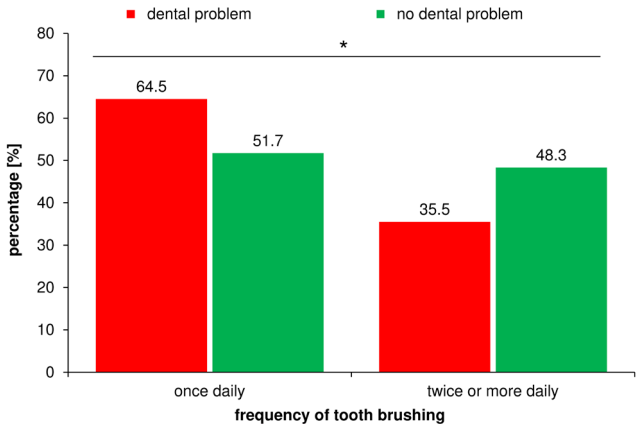

Fig. 8. Relationship between dental problems and the frequency of tooth brushing
* statistically significant ($p < 0.05$).

When asked about the measures taken to maintain their child's oral health, 221 (58%) parents stated that they did not have time for any additional methods. An equal number of parents added flossing or mouth rinsing ($n = 25$, 7% for each) to the oral hygiene routine of their child. The remaining parents increased the frequency of tooth brushing. Two hundred and seventy (71%) parents assisted their child during tooth brushing; however, there was no significant correlation between assistance at tooth brushing and the occurrence of dental problems during the pandemic ($p = 0.906$) (Fig. 9).

Dental information

Fortunately, in this survey, only 57 (15%) children experienced dental trauma during the pandemic, of which only 12 (21%) sought treatment at the dental clinic. One hundred and seven (28%) parents stated that they would take their child to the dental clinic for treatment during the pandemic, as advised by the dentist, and an approximately equal number of parents stated that they would take their child only for a dental emergency.

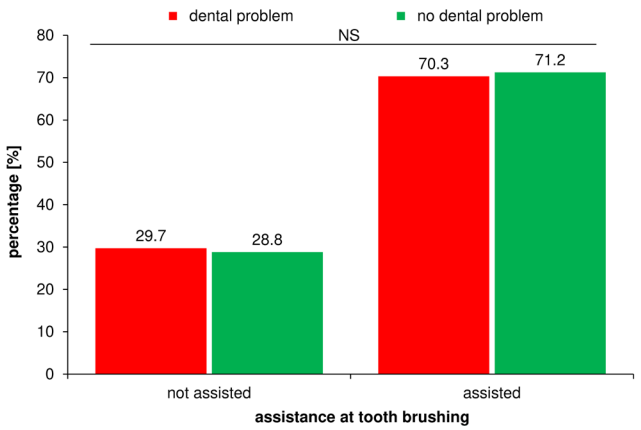

Fig. 9. Relationship between dental problems and assistance at tooth brushing
NS – non-significant.

Most parents (54%) were unsure about their child getting infected with COVID-19 during dental treatment. Only 14% were affirmative about their child getting infected at the dental clinic (Fig. 10). When asked about their preferred mode of contact with the dentist, 162 (43%) parents still preferred visiting a nearby dental clinic (Fig. 11).

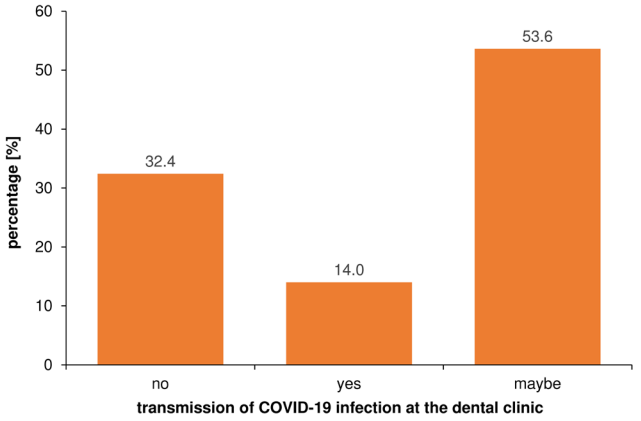

Fig. 10. Chances of the transmission of coronavirus disease 2019 (COVID-19) during dental treatment

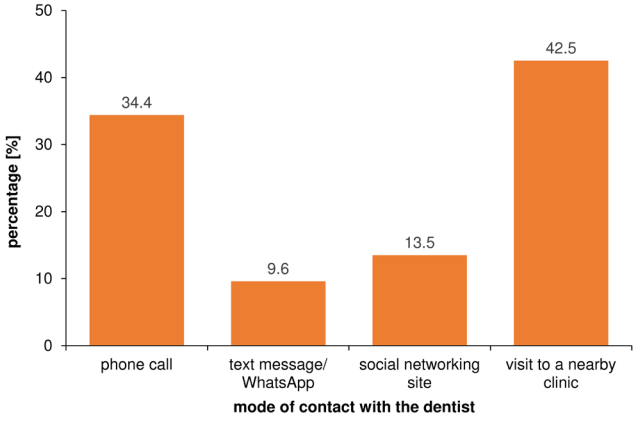

Fig. 11. Mode of contact with the dentist

Discussion

The COVID-19 pandemic has affected billions of people around the world, changing lives dramatically and requiring adaptations in all facets of life. Parents usually act as the primary caregivers for their child's general and oral health.[16] Good oral health is a prerequisite for the general well-being of the child. Parents should instill healthy dietary habits and good oral hygiene practices from an early age. Following up with constant guidance and supervision is essential to avoid severe oral health problems in the future.[17] The COVID-19 pandemic has created financial instability, unemployment and economic crises among many parents, which indirectly affects their mental health, and causes fear and anxiety. This can further lead to emotional distress and prevent the parent from monitoring their child's daily activities (e.g., oral hygiene practices).[3] Since parental attitudes and practices significantly impact the child's oral health, these variables were assessed in this study.

According to Kamalova et al.[12] and Goswami et al.,[18] more than 50% of children in the 4–7-year-old age group who reported to pediatric dental emergency departments during the COVID-19 pandemic had acute dental pain in primary molars. Insufficient prevention and the neglected treatment of the existing dental problems, as well as low parental care could be the reasons why children of this age group had significantly more oral health problems during the pandemic.[12] Most parents adopted a work-from-home lifestyle during the pandemic, and being in a busy period of life and work, the time spent caring for their children was compromised. This could be a possible explanation for the increased number of patients in this group reporting to the pediatric dental emergency departments during the COVID-19 pandemic.

The majority of parents in this study (58%) reported no changes in the amount of food consumed by their child during the pandemic. Campagnaro et al. stated that 61% of their respondents reported an increase in food intake during the pandemic,[3] which contradicts the findings of this study.

In a study by Goswami et al., 77% of the respondents increased their intake of high-carbohydrate foods.[10] The authors suggested that it could be attributed to new routines – work-from-home for parents and remote classes for children. In this survey, 43% of the children consumed more processed foods during the pandemic, which resulted in more dental problems.[10] The stockpiling of processed foods during the pandemic for the ease of use during and between meals could have possibly led to a higher consumption of these food items. These dietary changes have been shown to affect general health in the form of increased obesity and nutritional deficiencies, as well as an increased risk of dental caries.[3] However, this is contrary to a study by Kalyoncu et al., in which the mothers recognized the importance of oral health for the general well-being of the child; they provided non-cariogenic foods to their children and the children consumed healthier food items.[13]

The frequency of snack consumption during the COVID-19 pandemic was significantly higher in this study as compared to the results obtained by Goswami et al.[10] These dietary habits, as explained by Kotha et al., have been shown to be proportional to the decayed, missing due to caries and filled teeth (DMFT) score.[19] The presence of caries is related to the nature of food consumed and the frequency of eating. Parents need to be educated about the cariogenic and cario-protective properties of specific foods. Furthermore, they should plan to avoid the frequent consumption of high-carbohydrate foods.[17,20]

Eighty percent of the children in this survey used electronic devices at mealtimes and while snacking. This indirectly leads to poor diet quality characterized by higher fat and sugar consumption, with fewer fruits and vegetables, which is a potentially cariogenic diet. This would explain the strong correlation between the use of electronic devices and dental issues in this study.[21] Das et al. stated that a longer use of electronic devices during eating and snacking compromises the oral clearance of food.[22] This leads to the food pouching habit in the oral cavity during screen viewing, which acts as a predisposing factor for dental caries.[22] Such observations are similar to those made in this study.

The majority of parents in this study reported a once-daily brushing schedule for their children. However, Goswami et al. claimed that parents increased the frequency of tooth brushing due to constraints in achieving adequate dental services during the pandemic.[10] Our results are in agreement with a study conducted in Istanbul, in which the frequency of tooth brushing did not change.[13] In the present study, the results referring to the parents' assistance at tooth brushing during the pandemic were similar to those reported by Campagnaro et al.,[3] but contrary to the results of Kalyoncu et al., who reported fewer parents supervising their child's tooth brushing during the COVID-19 pandemic.[13] When asked about additional efforts made to maintain the oral hygiene of their child, the majority of parents in this study increased the frequency of tooth brushing, while a small percentage added mouth rinsing or flossing to the routine. However, most parents did not find enough time between their work schedules for implementing additional oral hygiene measures. These findings are consistent with a study by Goswami et al.[10] The prevention of caries is based on adequate and effective oral hygiene measures in addition to dietary habits.

According to some studies, children spent the entire day in a home setting, which results in the creation of active play patterns, and thus the risk of dental tissue trauma is increased. However, in this study, only 15% of children experienced dental trauma during the COVID-19 pandemic; this is similar to the incidences reported by Campagnaro et al.[3] and Kamalova et al.[12] This can be explained by the prohibition of outdoor games, sports training and coaching for children during the COVID-19 pandemic. In the present study, 21% of the children who experienced dental trauma sought treatment at the dental clinic, which

is contrary to the findings of Campagnaro et al., where only 17% of the respondents were willing to take their child to the dentist during the pandemic, regardless of the procedure.[3] However, the present study identified a slight increase (28%) in the number of parents who would follow the treatment protocol, as advised by the dentist. Considering the need for emergency treatment, the percentage of respondents who would seek urgent care was much higher in the Brazilian population as compared to this study.[3] A study conducted in Istanbul also showed a similar attitude of parents regarding dental care during the pandemic.[13] The reluctance of the parents in the present study to utilize pediatric dental care services may negatively impact the oral health of these children and trigger the need for extensive invasive treatment in the future.[3] Regular dental visits allow the detection of dental issues much earlier, which reduces the treatment time, and subsequently the treatment cost. With time, the approval of COVID-19 vaccines for young children will be underway; these may cause orofacial side effects, such as burning sensations, taste alterations, xerostomia, and pain, as reported in adults.[23] Though these symptoms are self-limiting, parents need to be aware of them and report back to the pediatric dentist for professional care.

Contrary to the results obtained in this study regarding parental views about their child being infected with COVID-19 at the dental clinic, Sun et al.[16] and Kalyoncu et al.[13] reported that more than 90% and 80% of the parents, respectively, agreed that their child could be easily infected with the virus during treatment. Respiratory viruses, such as SARS-CoV-2, can be transmitted through direct or indirect contact, or through small droplets. As pediatric dentists, we are potentially at a higher risk, since dental treatment involves aerosol-generating procedures. Thus, appropriate precautions need to be taken to prevent the virus from infecting children as well as its transmission from infected children to healthcare professionals.[13] There needs to be a focus on parental education regarding the measures taken for sterilization, disinfection and protection in the dental clinic.[24] Several authors have described the insufficient use of teledentistry, which is an effective consultation tool, along with safeguarding against the transmission of the infection during the pandemic,[10,25] which was also observed in this study. This reluctance might be due to the complexity involved, coordination issues or resistance to learn new skills. In the future, pediatric teledentistry could be useful for counseling children and parents with regard to appropriate oral hygiene measures.[17,26]

Limitations

This study has certain limitations. The most significant is that the study sample mostly belonged to the upper and upper middle class population, so we were unable to analyze variations in the results based on different socioeconomic statuses.

Conclusions

This study highlights the deficits in the attitudes and practices of the parents of 4–7-year-olds in relation to dietary habits, oral hygiene measures and the use of dental services during the COVID-19 pandemic. This could be attributed to a lack of awareness, the fear of exposure and the inconveniences faced by parents. The impact of the pandemic have been far-reaching for our children. It is essential to not underestimate the indirect effects of COVID-19 on the oral health of children.

Ethics approval and consent to participate

This study was approved by the Ethics Committee at the School of Dentistry of D.Y. Patil Deemed to be University, Navi Mumbai, India (IREB/2021/PEDO/21). Informed consent was obtained from all participants.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Importance of education on infection control and on the hand skin health of dental personnel

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Abstract

Background. Hand hygiene plays a significant role in infection control, yet it is performed correctly only 40% of the time. The daily use of soap, disinfectants and gloves can also affect hand skin health. Periodical educational interventions regarding hand hygiene can improve infection control.

Objectives. The current study aimed to identify the existing hand hygiene practices applied by dental personnel, to evaluate knowledge about infection control, to determine the adverse effects of hand hygiene on the skin, and to assess the effectiveness of the educational interventions concerning these topics.

Material and methods. This study was carried out at the Vilnius University Hospital Žalgiris Clinic, Lithuania. At the 1st stage, data was collected by using a self-administered questionnaire. At the 2nd stage, dental personnel underwent an educational intervention and the surveys were redistributed to determine any changes in the level of knowledge.

Results. In most cases, dental workers performed hand hygiene when it was needed. The proper method was selected by 53.4% on average. The main mistakes were the excessive use of soap and only occasional use of a disinfectant. The reported hand skin side effects included dryness (68.8%) and fissures (37.5%). Only half (50.5%) of the staff regularly used emollients. After the educational intervention, there was a 24.9% improvement in hand hygiene compliance.

Conclusions. The correct procedure for hand hygiene was reported by half of the participants. Washing hands with soap was the preferable choice, while alcohol-based hand rub (ABHR) was avoided. Skin problems were reported by more than 70% of the respondents. Training had a positive impact on the hand hygiene knowledge of the dental personnel.

Keywords: education, dentistry, infection control, hand hygiene, skin care

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Introduction

The human mouth contains one of the most diverse microbiomes in the human body. More than 1,000 species of bacteria are found in the oral cavity.[1] Regarding the recent COVID-19 pandemic, the issues of infection control and hand hygiene in the dentistry field have come to light. Dental personnel is exposed to an immense risk due to the specificity of dental procedures, which involve face-to-face contact with patients, frequent exposure to saliva, blood and other fluids, the handling of sharp instruments, and the generation of aerosols and droplets.[2] Although appropriate hand hygiene is a routine procedure in dental practice, the compliance rates are relatively low.[2] Data shows that proper hand hygiene is achieved only in 40% of cases.[3] Given the current situation, improvement in this area is of utmost importance.

The World Health Organization (WHO) has declared that hand hygiene is necessary before and after touching the patient, before handling invasive devices, after removing sterile or non-sterile gloves, and after contact with blood and other body fluids or inanimate surfaces and objects.[4] Data shows that hand hygiene is most often performed after exposure to organic fluids (98.5%), before invasive procedures (87.5%), before clean/aseptic/surgical procedures (83.1%), and between patients (81.4%).[5] However, proper hand hygiene after contact with patient surfaces is usually not achieved.[5] Such behavior increases the risk of cross-contamination between the patient and medical personnel.[6,7]

Hand hygiene includes cleaning the hands with soap and water and/or alcohol-based hand rub (ABHR). Indications for hand washing are as follows: before the first patient; after using a toilet; when the hands are visibly dirty or soiled with blood or other body fluids; or if there is no ABHR.[4] In all other clinical situations, dental personnel should use just ABHR. According to studies, ABHR is well-tolerated and safe, while the frequent use of soap increases the risk of developing a dermatological pathology.[8–10] Unfortunately, healthcare workers often ignore ABHR due to the erroneous belief that it provokes adverse skin effects, and use soap unnecessarily.[6] This often leads to the deterioration of the hand skin condition, including impaired barrier function, a change in the skin flora, increased bacterial shedding and sensitivity of the skin, or other adverse reactions.[10] Moreover, severe dermatological pathologies, such as contact and allergic dermatitis, can develop.[4,7,9]

The use of gloves is part of the standard precautions against cross-contamination in medical offices.[11] Latex gloves may cause allergic reactions because of various chemical agents (antioxidants, accelerators, activators, etc.) used during the manufacturing process or due to their protein content. Latex allergy occurs in 1–2% of the total population, but the sensitization rates in healthcare providers are 2 times higher than in the general population.[12] The main reasons for sensitization are 'wet work' and long working hours while using gloves.[13]

Hand hygiene interventions are described as hand sanitization programs to increase hand hygiene compliance. These interventions vary from simple (e.g., increased availability of cleaning agents) to complex (e.g., multimodal programs involving education, environmental restructuring, reminders, and performance feedback).[14,15] Single-component interventions improve hand hygiene practices, but multimodal strategies are more effective over a long term.[16] Education, feedback, support from a team leader, and the accessibility and visual reminders of hand hygiene are all elements that increase compliance.[16] Numerous studies have found positive effects on hand hygiene quality after educational interventions.[14–18] However, the compliance rates decline with time; therefore, regular post-interventions should be considered.[16]

The present study aimed to identify the existing practices reported by dental personnel for hand hygiene, to evaluate their knowledge about infection control, to determine adverse effects on hand skin, and to assess the effectiveness of the educational interventions concerning these topics.

Material and methods

The study was conducted in 2 stages. At the 1st stage, data was collected using a self-administered questionnaire. The questionnaire included multiple-choice questions about hand hygiene habits (daily cleaning with soap and ABHR), infection control knowledge (questions from the WHO's "Guidelines on hand hygiene in health care"[4]), hand skin health, the use of gloves, and the manifestations of adverse reactions, such as dryness or redness. The study included dental specialists, general dentists, dental resident doctors, and dental assistants/nurses working the day shift in the Žalgiris Clinic, Vilnius University Hospital, Lithuania. The day shift in the Žalgiris Clinic lasts from 8 a.m. to 2 p.m. or from 2 p.m. to 8 p.m. (in general, 6 h). Only 120 dental workers from the Žalgiris Clinic met these criteria, and all of them were given a first-stage questionnaire. After analyzing the results from the 1st stage, we assessed the most common mistakes. Accordingly, we prepared educational material and introduced it to the dental personnel at the 2nd stage. The instructional video material was uploaded to the Žalgiris Clinic intranet and informational posters were hung in each consulting room above the washing sink at eye level. A week later, the same questionnaires were redistributed among those who participated in the 1st phase of the study and saw the instructional video material to determine any changes that occurred after exposure to the educational intervention.

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics for Windows software, v. 23 (IBM Corp., Armonk, USA). Data normality was checked.

The χ^2 test of independence was used to determine statistically significant associations between 2 variables, and the non-parametric McNemar's test was used to assess changes between first- and second-stage responses. A statistically significant difference was assumed at 5% ($p \leq 0.05$).

Results

A total of 120 persons participated in the 1st phase of the study. The response rate was 80% (96/120). The majority of respondents were women (92.7%), and the age of the participants ranged from 20 to 66 years (39.02 ±11.17 years). For further analysis, the groups were divided according to specialization (nurses/dental assistants, resident doctors, dental specialists, and general dentists). The majority of respondents in both phases were nurses/dental assistants. In the 2nd phase, 96 individuals participated and the response rate was 87.5% (84/96). The majority of respondents were also women (89.3%), and the age ranged from 24 to 66 years (40.41 ±11.70 years) (Fig. 1).

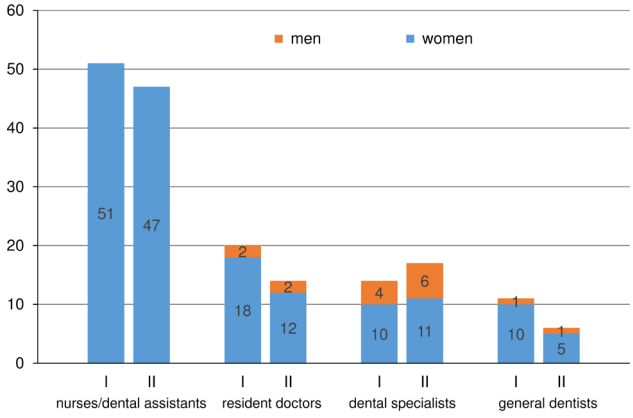

Fig. 1. Distribution of respondents by occupation and gender at the 1st and 2nd stage of the study

Knowledge about infection control

Nearly 100% of the respondents identified the situations where hand hygiene was necessary. However, the proper hand hygiene method (the use of soap and water and/or ABHR) was selected by only 53.4%. Table 1 shows to what extent correct hand hygiene measures were complied with at the 1st and 2nd stage of the study. At the 1st stage, 89.6% of respondents chose the proper hand hygiene method after exposure to organic fluids and 76.0% before invasive procedures. With regard to other measures, less than 34% replied correctly. After the educational intervention, a 21.4% improvement was observed in the selection of the proper hand hygiene method. However, at the 1st stage, when the respondents were asked to self-evaluate (on a 100% scale) to what extent they performed appropriate hand hygiene, a high rate of compliance was indicated (82.7%).

Eighty-three percent of the respondents correctly answered the question about the recommended hand washing time when using soap (Fig. 2). In addition, 79.8% knew the time required for the use of ABHR (Fig. 3).

At the 1st stage of the study, 47.9% of the respondents knew that during hand washing, the direction of water flow should be from the wrists to the fingers. After the educational intervention, a 25.9% improvement in this aspect of knowledge was observed (Table 1).

A difference in the opinions of the respondents regarding hand washing with soap and the use of ABHR at the 1st and 2nd stage was evident. After the intervention, the average improvement was 30.3% (Fig. 4).

Self-evaluation showed that 80.6% of the dental personnel believed they had enough knowledge about infection control and hand hygiene. A borderline statistically significant difference ($p = 0.049$) was observed between different professions, where 42.9% of general dentists indicated a lack of information as opposed to only 10.4% of nurses/dental assistants.

Table 1. Correct answers to the same questions at the 1st and 2nd stage of the study

Questions	Answers at the 1st stage $N = 96$	Answers at the 2nd stage $N = 84$	*p*-value
Appropriate hand hygiene before each patient	32 (33.3)	51 (60.7)	<0.001*
Appropriate hand hygiene after removing gloves	30 (31.3)	54 (64.3)	<0.001*
Appropriate hand hygiene after exposure to organic fluids	86 (89.6)	76 (90.5)	0.791
Appropriate hand hygiene before invasive procedures	73 (76.0)	71 (84.5)	0.700
Appropriate hand hygiene after using a toilet	27 (28.1)	26 (31.0)	0.607
Hands should be disinfected for 20–30 s	71 (74.0)	67 (79.8)	0.845
Water should flow from wrists to fingers	46 (47.9)	62 (73.8)	<0.001*
Soap dries skin more than ABHR	61 (63.5)	74 (88.1)	0.003*
Frequent hand washing with soap causes hand skin problems	72 (75.0)	67 (79.8)	1.000
Frequent use of ABHR does not cause hand skin problems	28 (29.2)	35 (41.7)	0.091

Data presented as number (percentage) (*n* (%)). ABHR – alcohol-based hand rub.

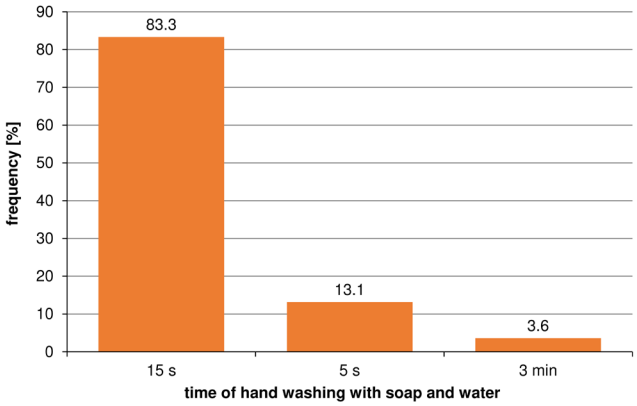

Fig. 2. Time required for hygienic hand preparation with soap and water

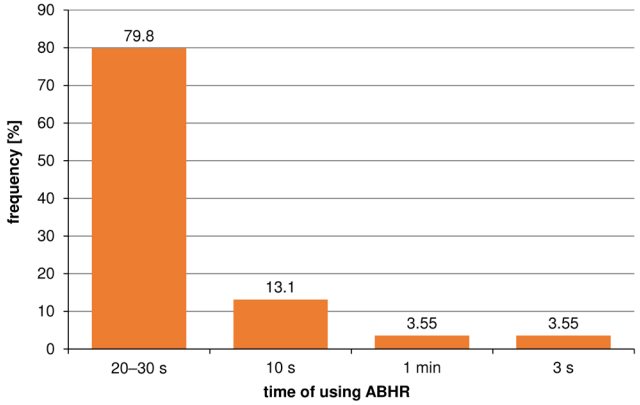

Fig. 3. Time required for hygienic hand preparation with alcohol-based hand rub (ABHR)

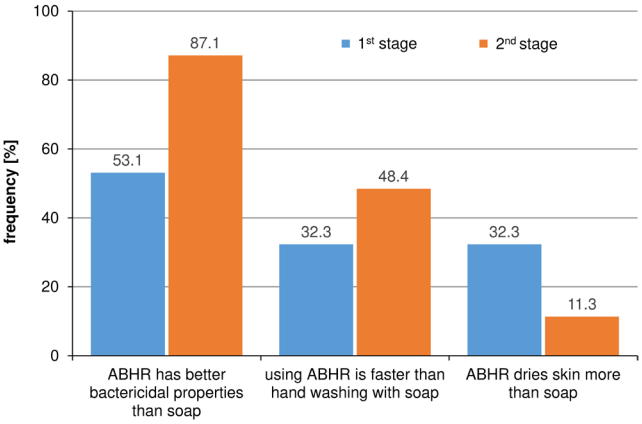

Fig. 4. Opinions on the use of soap and alcohol-based hand rub (ABHR) during hand hygiene procedures

Hand skin health

According to the self-reports, the prevalence of eczema and atopic dermatitis was 8.5% and 4.2%, respectively. Most often, the dental personnel indicated having a dry (45.8%) or mixed (32.3%) hand skin type.

A total of 50.5% of the respondents regularly used hand cream/lotion. However, only 38.5% used hand cream/lotion 3–4 times a day. Mostly emollients were used to counteract hand dryness (35.1%). The use of emollients after the educational intervention increased up to 77.4%, and a 26.9% improvement was evident. All healthcare workers who had atopic dermatitis ($p < 0.05$) and 50% of those who had eczema did not use hand skin emollients regularly. Only 19.8% of the respondents indicated that they did not have adverse hand skin effects, with the remaining participants specifying various complaints. There were no statistically significant differences in the distribution of various skin side effects within particular profession groups (Fig. 5).

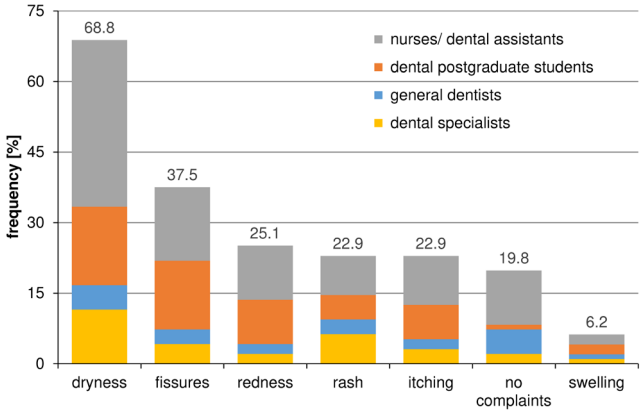

Fig. 5. Distribution of adverse hand skin reactions according to different professions

Use of gloves

A total of 62.4% of the respondents wore gloves for 7 h or more, 36.6% for 3–6 h, and 1.0% for up to 2 h. Half of the respondents indicated working with gloves for 10 years or more.

During the day, 57.3% changed their gloves 11 times or more, 34.4% 6–10 times, and the remaining 8.3% up to 5 times. Of those who changed their gloves up to 5 times (n = 8), 62.5% (n = 5) had 6–10 patients per day, and of those who changed their gloves 6–10 times (n = 33), 9.1% (n = 3) had 11 or more patients per day.

Most often, nitrile (47.7%) or latex (46.7%) gloves were used. Approximately 1/3 of the participants (27.4%) reported irritation reactions linked to the wearing of latex gloves, 6.4% to nitrile gloves and 6.3% to vinyl gloves.

Effectiveness of the educational intervention

The answers to the same questions were compared between the 1st and the 2nd stage. The responses to 4 out of the 10 questions were significantly improved after the educational intervention (Table 1). The average improvement after educational training was 25.4%.

Most respondents (95.2%) agreed that educational training had an impact on their knowledge. A total of 54.75% of the healthcare workers indicated that infection control and hand hygiene training should be carried out every year, 29.75% indicated every 6 months, and 15.5% every 3 years or more.

Discussion

One of the critical issues in hand hygiene is recognizing situations when particular hand preparation is required.[4] Medical personnel tend to forget about hand hygiene when contact with patient surfaces is expected as well as after it.[5] This study found that the compliance rates declined after the removal of gloves, but the results were much better after the educational intervention. After such an intervention, appropriate hand hygiene was performed by 100% of the personnel in all other situations (after contact with organic fluids, before invasive procedures, before each patient, and after using a toilet). Although dental workers self-evaluated their knowledge as very good, the answers about the correct method to use in different situations revealed that their knowledge was inadequate. At the 1st stage, only half of the staff knew which situations required washing hands with soap and/or ABHR.

Sodium lauryl sulfate (SLS) is a detergent commonly used in soap due to its cleansing properties. However, it affects the natural skin barrier and causes skin dryness, irritation and allergic reactions.[19] Also, it is widely known that SLS irritates the skin more than ABHR.[9] For this reason, the use of soap should be avoided in situations that do not require it. In this study, the majority of dental personnel washed their hands with soap before each patient and after removing gloves, while these situations require only the use of ABHR. In another study, 68% of respondents reported that they washed their hands with soap between patients and 65% after removing gloves; in contrast, only 3% used ABHR between patients and 6% after removing gloves.[20] After the educational intervention, improvement in choosing the proper hand hygiene method was evident, and fewer workers believed that ABHR dried skin to a greater extent than soap.

Another essential aspect concerning hand hygiene is the adequate application time for soap and ABHR. Using ABHR for less than 15 s does not destroy certain pathogens, while using it for more than 15 s is not contraindicated, as despite its enhanced antiseptic properties, ABHR does not irritate the skin.[21] On the contrary, in the case of soap, a long application time is not recommended, as soap contributes to hand skin dryness.[9] One study found that the majority of health professionals washed their hands with soap for 15 s or longer, while others chose a shorter time.[22] In the present study, the majority of dental personnel washed their hands with soap for the recommended time (15 s), but some selected a longer duration of 3 min. Most of the respondents followed the recommended time for hand disinfection with ABHR.

It is well known that during hygienic hand preparation, water should flow from the wrists to the fingers. Washing in this manner allows water to flow from the least contaminated area (the wrists) to the most contaminated parts (the fingers).[4] In the first-stage questionnaire, only half of the respondents answered this question correctly. It might be due to the fact that some respondents confused this question with surgical hand preparation. This aspect was mentioned in the educational video material and a significant improvement was achieved.

There is no doubt that the daily use of soap and disinfectants, working with gloves, and 'wet work' affect hand skin health. A European population survey showed that 54.1% of the population had a dry hand skin type.[23] In the present study, nearly half of the respondents also had dry skin. This type of skin is even more vulnerable to irritants, and thus side effects can appear quickly.[23] In a study by Harnoss et al., the most common adverse hand skin reactions among surgeons of various specialties were dryness, nail splitting and cracking, itching, and redness between the fingers, whereas 49% of doctors reported no complaints.[24] In this study, the most common side effect was also dry hand skin, but it occurred almost 5 times more often, while the response 'no complaints' was noted 2.5 times less frequently than in the abovementioned study. Both studies had different population samples. In the first one, most of the respondents were men and all of them were surgeons, for whom there are specific hand hygiene requirements. In our study, there were more women, and all the respondents were dental personnel.

Due to specific working conditions, dental personnel are at high risk of developing a dermatological pathology.[9] The incidence rate of eczema varies in the literature. The 1-year prevalence among dentists in Japan was 36.2%.[9] In healthcare workers in Denmark[25] and Sweden,[8] the prevalence was 21%, and in Dutch healthcare workers it was 12%.[26] The prevalence rate in the present study was less than 9%, but this may have been due to the small-scale study population, and also to the fact that no objective tests were carried out to confirm the diagnosis. The age of patients with eczema was lower than in the general study population, and the same tendency has been observed by other researchers.[9,26] One possible explanation for this finding is that eczema tends to develop at the early stages of working, and health workers who are severely affected may leave their profession at a young age.[9] The prevalence of another common disease – atopic dermatitis – also varies. In Japan, its incidence among dental practitioners was 15.8%,[9] and in healthcare workers in Denmark and Sweden, the incidence was 14.5%[8] and 22%,[27] respectively. The prevalence of atopic dermatitis in this study was also low (4.2%).

Latex allergy is an occupational hazard for medical personnel. According to various studies, latex allergy affects about 4% of healthcare professionals.[28,29] In one study, 41.4% of nurses experienced irritation reactions caused by latex gloves.[30] Nurses who reported an irritation reaction were given a skin prick test and 9.8% of them were diagnosed with latex allergy. In the present study, the prevalence of an irritation reaction related to wearing latex gloves was 27.4%. As a skin prick test was not performed, no verified data on latex allergy was available.

The regular use of skincare products could help to prevent dermal side effects in healthcare workers. The protective action of such products most likely comes from 2 different components of the cream (oil and wax), which prevent, to some extent, the evaporation of epidermal water, and polyalcohols, such as glycerol and propylene glycol, which have moisturizing properties.[31] It is recommended to use hand lotion or cream after each hand washing.[31] Alcohol-based hand rub should be used no sooner than 5 min after lotion application.[32] However, for various reasons, the use of emollients among medical personnel varies. According to some studies, 25% of surgeons and 91% of nurses use hand cream/lotion.[24,33] Regarding the use of handcare products, emollients are usually used when the skin is dry, before and after work, or randomly during the day, and rarely before and after each patient.[24] The same tendencies were evident in this research; skincare products were used irregularly only by half of the staff. After the educational intervention, the use of emollients increased significantly.

An important aspect of this study was the educational intervention. The most common mistakes from the 1st stage were identified and targeted for improvement in the intervention. This study demonstrated that it is unnecessary to repeat all information, and that only targeted data can be provided. According to Dale's Cone of Experience, humans remember only 10% of the information they read and 30% of what they see.[34] That is why the current educational intervention used a poster (readable information) and instructional video (visual communication) material. A crucial and novel aspect is that after receiving the selected information, the respondents answered the questions about hand hygiene and use of the hand hygiene methods in different situations much more accurately, and a general improvement was observed. Therefore, this study demonstrates that repeating targeted information is sufficient to achieve better knowledge. Moreover, it can be predicted that the workers adapted this knowledge into practice, and that hand skin health also improved.

Overall, the current data suggests that educational interventions are effective and lead to improved hand hygiene practice.[14–18,35] A recent systematic review concluded that multimodal training approaches raised hand hygiene compliance from a baseline of 51.5% to 80.1%.[18] Another long-term study found that before training, hand hygiene was performed 63.6% of the time, and after 8 years of monitoring and multiple interventions, it improved to the level of 84.4%.[35] The researchers also noted that due to this improvement, less hospital-related infections occurred.[35] The results of this study confirm that educational interventions can improve knowledge about the hand hygiene practice and infection control principles.

Conclusions

The dental personnel performed hand hygiene correctly in 53.4% of cases. Generally, the hands were washed with soap too often, while ABHR was avoided. Only 19.8% of the respondents never experienced skin problems. Training has a positive impact on hand hygiene and improves the knowledge of dental personnel.

Ethics approval and consent to participate

The study was approved by the institutional Ethics Committee at the Žalgiris Clinic, Vilnius University Hospital, Lithuania (No. S(5.2.)-716). Written informed consent was obtained from all participants.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Comparison of the methods of disinfection/sterilization of extracted human roots for research purposes

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Conflict of interest
None declared

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Abstract

Background. Extracted human teeth are used to simulate dental procedures and are essential for practical education and research studies.

Objectives. The aim of this study was to evaluate the efficacy of different sterilization methods for extracted human roots and to assess the effects of these methods on dentin microhardness.

Material and methods. The crowns of 40 mandibular incisors were removed. The roots were sectioned at 10 mm and divided into 4 groups ($n = 10$ per group): G1 – no sterilization (control); G2 – microwave radiation (650 W, 5 min); G3 – ethylene oxide (288°C, 3 h); and G4 – autoclave (121°C, 15 min). The roots were immersed in brain heart infusion (BHI) and incubated at 37°C in variable oxygen atmospheres. After 14 days, the samples were assessed for turbidity. Three slices were obtained from each root, and indentations were made at 30, 60 and 120 µm from the root canal lumen. The microbiological data was analyzed with the Kruskal–Wallis test and Dunn's post-hoc test. Microhardness was evaluated by means of the two-way analysis of variance (ANOVA) and Tukey's test ($p < 0.05$).

Results. The roots submitted to autoclaving were 100% sterile, which differed from the other methods ($p < 0.05$); the control specimens had 0% sterility. For microhardness, significant differences were found between the methods, particularly for the apical third (68.06 ±12.50) ($p < 0.05$).

Conclusions. Although all the evaluated techniques reduced dentin microhardness, autoclaving should be used as the most reliable method of sterilization of extracted dental roots.

Keywords: hardness test, autoclave, ethylene oxide, microwave oven, tooth root

Introduction

Extracted human teeth are used to simulate dental procedures and are essential to practical education and research studies.[1–3] In vitro and in situ studies provide scientific support for developing new materials, and allow the control and simulation of real situations in a laboratory environment.[4,5]

The American Dental Association (ADA) and the Centers for Disease Control and Prevention (CDC) recommend a thorough removal of microorganisms capable of transmitting diseases from non-disposable materials used in patient care.[1,6,7] The effective elimination of microorganisms is necessary to perform in vitro and in situ research in an attempt to maintain the substrate structure unaltered.[7–10] This objective is hard to achieve due to the abundance of microorganisms present in the oral cavity, biofilm and saliva.[2,7] Therefore, there is a high risk of infection and cross-contamination when handling extracted teeth.[2,7–9]

Disinfection refers to an action that reduces the microbial load present on the surface of an object, whereas sterilization refers to a process that removes any detectable microbial load.[11,12] Various chemical sterilants have been tested for the disinfection of extracted teeth, including formalin, glutaraldehyde, iodopovidone, alcohol, vinegar, thymol, and chloramine.[3,12] The literature also reports the use of physical agents, autoclaving, gamma radiation, microwave radiation,[2,8,13] and gases, such as ethylene oxide and formaldehyde,[11] to sterilize extracted teeth.

The effectiveness of any method of sterilization depends upon time, contact, temperature, pressure, and how the specimens are stored, washed and decontaminated.[9–12] Autoclaving consists in using pressurized steam to achieve the hydrolysis and coagulation of cellular proteins; it kills bacteria, viruses, spores, and all other microbial forms.[3,8] It is recommended to follow the manufacturer's protocol and never interrupt the sterilization cycle. After the process, the sterilizer must be depressurized and the packs must remain inside for drying.[11]

The process of sterilization in ethylene oxide steam alternates between ethylene oxide gas cycles and ventilation periods.[14,15] Microwave radiation is initially effective; however, depending on the time of exposure, cellular disintegration may occur due to an increase in temperature.[13,16] There have been concerns about the effects of infection control on extracted teeth, namely on dentin permeability and the bond strength of restorative materials.[1,17] It is unknown whether autoclaving affects the chemical and micromechanical relationship between dentin and dental materials.[9] Studies have shown changes in the chemical properties of dentin and a reduction in its microhardness.[2,18,19]

Another important consideration is that studies that evaluated the microbiological effectiveness of sterilization methods used small sectioned dental slabs,[2] which fails to simulate the real conditions of in situ studies. The standardization of the experimental design and of the teeth are required to produce valid results in laboratory research.[20–22] Previous investigations did not evaluate the growth of microorganisms under different oxygen incubation conditions (e.g., aerobic and anaerobic).[6,7,12] Conversely, this study used different oxygen environments in an attempt to eliminate every type of microorganism present.

Considering the aforementioned facts, it is important to investigate the effectiveness of sterilization methods for large dental slabs, such as roots, before using them for educational or research purposes. This study aimed to evaluate the efficacy of different sterilization methods that are commonly used for extracted human dental roots as well as their effects on dentin microhardness. The null hypotheses were: 1. There is no difference in sterilization effectiveness between the methods tested; and 2. There is no difference in dentin microhardness after using the different sterilization methods.

Material and methods

Sample preparation

Forty freshly extracted human incisors were stored in 0.1% thymol solution at 4°C. They were cleaned and examined to verify that there were no structural anomalies and that they had complete root formation. A radiographic examination was conducted to confirm that there was a single root canal in each tooth. The 40 teeth had their crowns removed (IsoMet™ 1000; Buehler, Lake Bluff, USA) and the roots were trimmed coronally to a standardized length of 10 mm. The final dimensions were checked with a digital caliper (Mitutoyo, Suzano, Brazil).

Dental root sterilization

The roots were divided into 4 groups (n = 10 teeth per group):

– G1 (control): no sterilization method was used;
– G2 (microwave): the specimens were placed in glass containers, individually immersed in 100 mL of sterile distilled water and exposed to microwave radiation in a domestic oven (Electrolux, Pinhais, Brazil) at 650 W for 5 min;
– G3 (ethylene oxide): the specimens were submitted to 50–80% relative humidity at 288°C for 3 h (Wimac Kliniekdiensten, Rotterdam, the Netherlands); and
– G4 (autoclave): the specimens were stored in a glass vessel containing distilled water and autoclaved at 121°C with 15 psi for 15 min in an industrial autoclave (model AG 523; Ortosintese Indústria e Comércio, Jaragua, Brazil).

All roots were immersed in sterile distilled water before and after sterilization to equally corrode the dental substrates.

Broth turbidity analysis

The roots from each group were individually immersed and transferred to test tubes containing 10 mL of sterile brain heart infusion (BHI). Each tube was agitated for 1 min in an agitator (Vortex, São Paulo, Brazil). For each experimental group, 3 tubes with the BHI broth and the roots (n = 12) were incubated under anaerobic conditions. Another 3 tubes for each group (n = 12) were incubated in a microaerobic atmosphere. Finally, 4 tubes for each group (n = 16) were incubated in an aerobic atmosphere at 37°C. In this way, it was possible to evaluate the growth of every possible type of microorganisms – aerobic, facultative anaerobic and anaerobic.

After a 14-day incubation period, the presence or absence of turbidity was assessed. Turbidity in the sample was regarded as the evidence of microbial growth in the broth; no visible growth in the broth was considered effective disinfection. The broth turbidity analysis is shown in Fig. 1.

Microhardness test

The specimens were mounted on a low-speed diamond machine (IsoMet 1000). Two slices of 2-millimeter thickness were obtained from each third of each root. The 1st slice of each third was used to determine microhardness.

The specimens were ground wet with 600- and 1,200-grit silicon carbide papers, polished with felt disks, and embedded in aluminum oxide paste at a low speed. Then, they were washed under running water, dried with gauze and examined at ×40 magnification to confirm smoothness. Dentin microhardness was measured with a Knoop indenter (HMV-2; Shimadzu, Barueri, Brazil) under a load of 25 g at a dwell time of 10 s. For each slice, 12 indentations were made (3 at each quadrant) at each depth – 30, 60 and 120 µm from the root canal lumen. The means for the quadrants were calculated.

Statistical analysis

The microbiological data was evaluated with the Kruskal–Wallis test and Dunn's post-hoc test. The microhardness data was evaluated according to normal and homogenous distribution (the Shapiro–Wilk test and Levene's test, respectively), followed by the two-way analysis of variance (ANOVA) (with regard to the sterilization methods and the root thirds) and Tukey's test. The analyses were performed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA), at a 5% significance level.

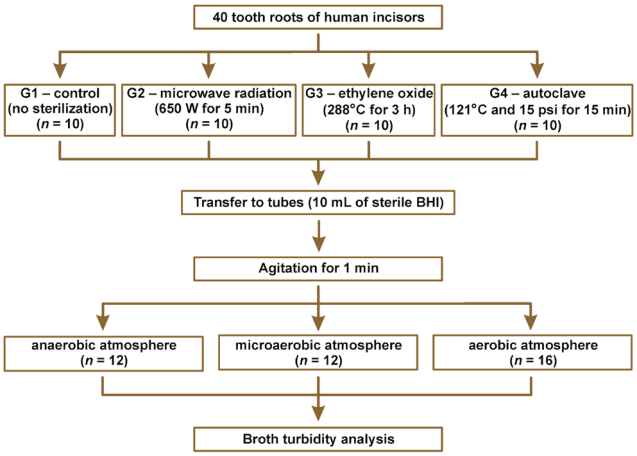

Fig. 1. Diagram of broth turbidity analysis
BHI – brain heart infusion.

Results

Broth turbidity analysis

The 1st null hypothesis of this study was rejected. The statistical analysis revealed significant differences between the sterilization methods. The roots submitted to autoclaving were 100% sterile (100% of samples were non-turbid), which was different from the other methods ($p < 0.05$). Ethylene oxide had 50% sterility. The control group (no sterilization) had 0% sterility; it was statistically similar to microwave radiation, which produced only 10% sterility ($p > 0.05$).

The patterns of turbidity, sterility, and the mean values for each group are presented in Table 1 and illustrated in Fig. 2. Figure 3 shows the samples after the experiment.

Table 1. Broth turbidity after 14 days per visual analysis and the percentage of root sterility for each experimental group (n = 10)

Groups	Broth turbidity†	Sterility [%]	*Me*	*Z*-value	*M*
Microwave radiation	(9/10)	10	2.0	1.87	1.9[a]
Ethylene oxide	(5/10)	50	1.5	–0.62	1.5[b]
Autoclave	(0/10)	100	1.0	–3.75	1.0[c]
Control	(10/10)	0	2.0	2.50	2.0[a]

Me – median; *M* – mean. † Broth turbidity indicates the presence of microorganisms. Different letters in superscript indicate statistically significant differences between the groups (Kruskall–Wallis test ($p = 0.001$) and Dunn's post-hoc test ($p < 0.05$)).

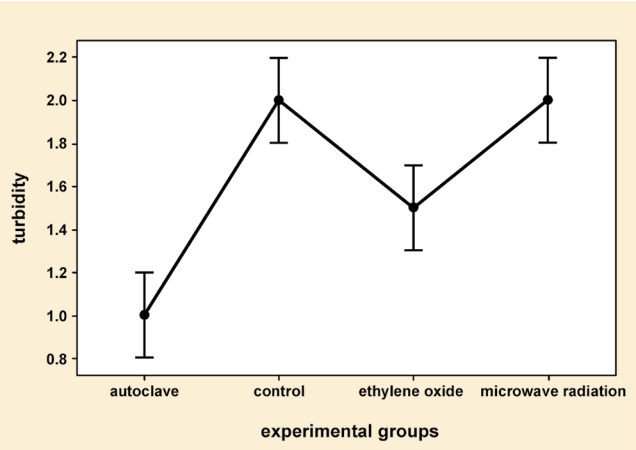

Fig. 2. Microbiological data for each experimental group
Data presented as median (*Me*) and 95% confidence interval (*CI*).

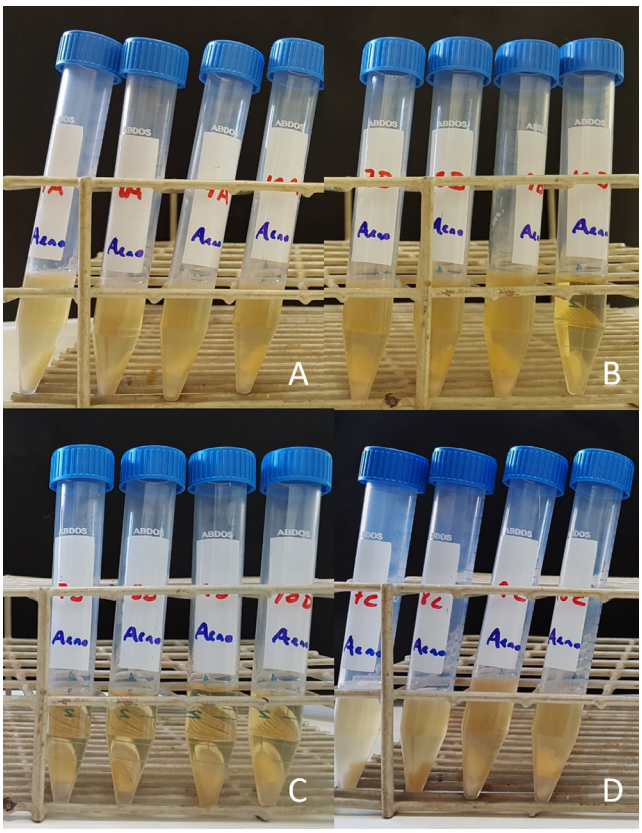

Fig. 3. Roots individually stored in tubes containing brain heart infusion (BHI), showing clear and turbid samples
A – microwave radiation; B – ethylene oxide; C – autoclave; D – control (no sterilization).

Microhardness test

The 2nd null hypothesis of this study was rejected, as some of the tested protocols significantly reduced dentin microhardness (expressed as Knopp hardness number (KHN)) in human root canal dentin. The two-way ANOVA revealed significant differences in the microhardness values between the sterilization methods ($p < 0.0001$), the root thirds ($p = 0.0041$), and with regard to the interaction of the 2 factors ($p = 0.0265$). The control group showed the highest mean value (91.09 ±4.08[a]), which was statistically significantly different from those for the microwave radiation (66.69 ±9.43[b]), ethylene oxide (71.42 ±9.61[b]) and autoclave (73.53 ±15.02[b]) groups. The highest microhardness values were found in the cervical (77.73 ±10.48[a]) and middle (81.25 ±15.26[a]) thirds ($p > 0.05$). The lowest values were found in the apical third (68.06 ±12.50[b]). The microhardness mean and standard deviation (*M* ±*SD*) values for each experimental group are detailed in Table 2.

Concerning the interaction of factors, the ethylene oxide, autoclave and control groups showed the highest microhardness values in the cervical and middle thirds ($p < 0.05$), whereas the microwave radiation group showed the lowest values in the middle third.

Discussion

In the current coronavirus disease 2019 (COVID-19) context, worldwide concern has arisen due to the risk of cross-contamination. Dental practice, education and research are some of the most highly impacted fields.[23] The oral cavity linked with the respiratory tract is a natural habitat for many microorganisms, including several bacterial species, several potentially pathogenic fungi, and viruses, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[24,25]

To reduce the risk of transmitting blood pathogens, tooth roots must be biologically safe before they are used. At the same time, they should have their normal mechanical properties maintained.[5,7,26] However, taking into account bacterial colonization, the tubular invasion of bacteria[27] and the complex microstructure of the teeth, tooth samples are difficult to sterilize, as sterilization measures can modify the tooth properties, and even a minor change can affect the results of the analysis.[3]

Table 2. Microhardness [KHN] according to the different methods of sterilization and root thirds

Root third	Microwave radiation	Ethylene oxide	Autoclave	Control
Cervical	77.57 ±19.74^{Ba}	75.25 ±29.40^{Ba}	66.42 ±22.49^{Ba}	91.70 ±4.65^{Aa}
Middle	60.85 ±18.75^{Ba}	78.52 ±20.96^{Ba}	90.79 ±19.29^{Ba}	94.83 ±7.94^{Aa}
Apical	61.65 ±8.71^{Bb}	60.49 ±23.10^{Bb}	63.38 ±9.68^{Bb}	86.73 ±10.13^{Ab}

Data presented as mean ± standard deviation (*M* ±*SD*).
Different uppercase letters in superscript indicate statistically significant differences between the sterilization methods ($p < 0.05$). Different lowercase letters in superscript indicate statistically significant differences between the root thirds ($p < 0.05$).

Previous authors have already tested the sterilization methods assessed in this study, and these protocols are commonly used in health services and research.[8,12,15]

Broth turbidity is a widely used test that is simple to perform. When correctly conducted, the turbidity measurements guarantee the quality and accuracy of the results.[6,28] Contrary to other studies,[6,7,12] we evaluated the growth of all types of microorganisms (aerobic, facultative anaerobic and anaerobic) to better simulate clinical conditions.

Mandibular incisors were used in this study due to their smaller size and thickness as well as lower anatomical variability as compared to other dental groups.[5] Furthermore, to the best of our knowledge, this is the first investigation to use 10-millimeter-long contaminated roots and not only small dental slabs.[2] This study aimed to support in situ research using acrylic palatal devices with human roots to allow biofilm accumulation.[5]

The 1st null hypothesis of this study was rejected. The outcomes revealed differences among the sterilization methods with respect to the presence of viable microorganisms. The roots submitted to autoclaving were 100% sterile; thus, this was the only method that was able to completely eliminate microorganisms from the extracted roots. The autoclaving process is the method recommended by ADA and by previous studies for materials exposed to body fluids.[1,6,7] Its main advantages are its rapidness and efficiency, excellent reliability, and cost-effectiveness. The limitation is that this method cannot be used with sensitive equipment and materials.[11] Autoclave sterilization is recommended for preventing cross-contamination during laboratory bonding tests with human teeth.[4] Talge Carvalho et al. stated that the autoclave sterilization of teeth slabs produced no significant changes in dentinal tubule morphology or dentin chemical composition.[28] Western and Dicksit considered autoclaving to be 100% effective and reliable.[29] However, extracted teeth with amalgam restorations should not be autoclaved because of mercury release.[30]

Concerning ethylene oxide sterilization, the literature reports that it provides powerful gas penetration and is the gentlest for delicate materials.[31] However, it is expensive, toxic, explosive, and requires long time cycles and a specialized aeration chamber.[11] For these reasons, it is better for hospitals and not practical for dental clinics. Ethylene oxide destroys microorganisms by chemically reacting with nucleic acid, and interfering with cellular metabolism and reproductive processes, which renders the affected microbes nonviable.[32] The results of the current study showed lower sterility than expected (50% of the roots were sterile), which is probably due to the parameters used. Other authors have also warned about the inability of ethylene oxide to sterilize human teeth.[4,15] Thomas et al.[14] found a significant interaction between the depth of caries lesions in enamel and ethylene oxide sterilization in vitro.[14] Ethylene oxide is only 20–36% effective against *Bacillus subtilis* in extracted teeth.[15] The literature does not provide any evaluation of the ethylene oxide sterilization of human teeth with the consideration of different equipment parameters.

Microwave radiation has been indicated for the disinfection/sterilization of laboratory materials.[31] However, few studies have assessed its ability to reduce the microbial load on dental substrates.[13,33] In the current study, microwave radiation was not a reliable method for sterilizing human roots. The mechanism by which microwave radiation kills microorganisms is not entirely understood. Microwaves promote decontamination by both thermal and nonthermal processes, since the electromagnetic field induced by microwaves causes biological and chemical alterations in cells.[13,33] Therefore, microwave radiation is generally used as a disinfectant, but it is ineffective for use with extracted teeth in laboratory research.

Microhardness measurement is one of the simplest methods of non-destructive mechanical characterization.[2] Dentin and enamel microhardness depend on the amount of mineral structure content, and provide indirect evidence of mineral loss or gain in hard dental tissues.[2] The mineral content of dentin decreases with age, which leads to dental fragility.[17]

The 2nd hypothesis of this study was rejected, since dentin microhardness was reduced after conducting the different sterilization processes. These results are consistent with those of previous studies, in which the sterilization methods also decreased microhardness.[2,8,9,18] This effect has been associated with calcium loss, which results in dentin demineralization and softening. It is hypothesized that the high temperature and pressure during sterilization disrupt collagen and denature the organic component in dentin, which affects microhardness.[1,2,29]

Reduced microhardness values may influence the results of studies that assess mechanical properties, such as bond strength, of resin-based materials.[1,8,17–19] Moreover, the sterilization process might increase the risk of tooth fracture, which is unwanted in preclinical endodontic training.[17] Other authors measured the surface microhardness, roughness and bond strength of denture teeth and acrylic resin denture bases, and verified the decrease in material microhardness; this outcome was considered a common and standard consequence of all sterilization methods.[27,34]

Finally, it is important to emphasize that infection control is not limited to the disinfection/sterilization of extracted teeth. Students and researchers should wear gloves, masks and eye protection when handling extracted teeth.

In this study, the best sterilization method for eliminating microorganisms was autoclaving, even though it reduced microhardness. The choice of method depends on particular situation, analysis and the study objective. For in situ and in vitro research with human teeth, we recommend autoclave sterilization before the laboratory steps. Further studies with additional methods should be conducted to determine sterilization methods and protocols that cause the least alteration of tooth mechanical properties.

Conclusions

Based on the experimental methods and results of this study, it can be concluded that autoclaving is the most effective sterilization method to eliminate microorganisms from 10-millimeter-long human roots. Regardless of the method tested, the roots submitted to disinfection/sterilization had their dentin microhardness reduced.

Ethics approval and consent to participate

This study was approved by the institutional Ethics Committee at the Ribeirão Preto Dental School, University of São Paulo, Brazil (No. of approval: 37032114.1.0000.5419).

Data availability

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

Consent for publication

Not applicable.

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Original paper

Altered immunoexpression of SOX2, OCT4 and Nanog in the normal-appearing oral mucosa of tobacco users

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Abstract

Background. Tobacco use is causatively associated with various human cancers, including oral carcinoma. A number of pathways have been delineated to describe its etiopathological link with oral carcinogenesis, including alterations in the expression of stem cell markers. Embryonic stem cell markers, such as sex-determining region Y-box 2 (SOX2), octamer-binding protein 4 (OCT4) and homeobox protein Nanog, which are mainly involved in the maintenance of stemness and pluripotency, have been positively associated with the pathogenesis of oral potentially malignant disorders and oral cancers. In this context, we attempted to explore the subcellular impact of tobacco through examining the expression of these stem cell markers in normal and normal-appearing oral mucosa in non-tobacco users and tobacco users.

Objectives. The aim of the study was to analyze the immunoexpression of SOX2, OCT4 and Nanog in the normal-appearing oral mucosa (NAOM) of tobacco users as compared to the normal oral mucosa (NOM) of non-tobacco users.

Material and methods. The tissue samples of tobacco users and non-tobacco users ($n = 50$ per group) were immunohistochemically stained to assess the expression of SOX2, OCT4 and Nanog.

Results. In the oral mucosa of non-tobacco users, a peculiar parabasal expression pattern of SOX2 and OCT4 was observed, whereas Nanog was non-reactive. The grade of inflammation was found to be a predictive variable influencing the expression of the 2 markers. In tobacco users, variables such as male gender, mixed habit and basilar hyperplasia significantly controlled the basilar and suprabasilar expression of SOX2, OCT4 and Nanog. The expression of SOX2 and OCT4 was higher in tobacco users; in particular, OCT4 positivity was significantly increased ($p < 0.001$) in comparison with non-tobacco users.

Conclusions. The altered expression of the examined stem cell markers could be an indication of early molecular changes in NAOM under the influence of tobacco.

Keywords: tobacco, stem cells, oral mucosa

Cite as
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Introduction

Tobacco use is one of the leading contributors to untimely deaths and associated economic damage globally, as it has been consistently linked with the etiopathogenesis of several human cancers, including oral cancer. Various forms of tobacco, including smoking and smokeless, or some combination of both, are thought to be involved in the multistep process of oral carcinogenesis.[1] Around 60 chemicals, including pro-carcinogens that are present in tobacco products, can cause genetic and epigenetic aberrations in oral mucosal cells through DNA damage and chromosomal instability, and thus contribute to field cancerization. These subcellular changes appear to be early events in multistep progression from clinically normal and histopathologically non-dysplastic mucosa (normal oral mucosa (NOM)), through oral potentially malignant disorders, to oral cancer.[2] The deleterious effects of tobacco at the cellular level need to be detected early through biomarkers so that individuals at risk of developing clinical lesions can be identified and managed accordingly.

A well-characterized transcription factor called sex-determining region Y-box 2 (SOX2) is known to play a crucial role in the maintenance of pluripotency and the self-renewal capability of human embryonic stem (ES) cells.[3] Another transcription factor, octamer-binding protein 4 (OCT4) – a member of the POU-family of DNA-binding proteins, also contributes to the regulation of pluripotency of ES cells through cooperative interactions with SOX2.[4] Homeobox protein Nanog is another member of core pluripotency regulators that acts on the downstream targets of OCT4, and hence plays a key role in cell fate determination, mainly in the differentiation of ES cells.[5] Several clinical studies have reported on the association of these master regulators of ES cell pluripotency at various stages of carcinogenesis and the overall prognosis of various human malignancies.[6–8] The expression of SOX2, OCT4 and Nanog have been studied in combination with or without other stem cell markers as predictors of the risk of malignant transformation in oral potentially malignant disorders and with regard to the clinical outcomes of oral cancer. Despite the conflicting results, the overexpression of these transcription factors has been observed to play a vital role in oral carcinogenesis.[9–11] Furthermore, a number of studies have also noticed an increased expression of these markers in normal-appearing oral mucosa (NAOM) adjacent to the lesional carcinoma tissue, supporting the association of SOX2, OCT4 and Nanog in the field cancerization of oral mucosa under the influence of tobacco.[9,12] Based on this evidence, we evaluated the expression of SOX2, OCT4 and Nanog in the NOM of non-tobacco users in comparison with that of tobacco users by means of immunohistochemistry to assess the influence of tobacco on oral mucosa prior to any evidence of clinically established lesions.

Methodology

Patients and tissue sample collection

The research protocol was reviewed and ethically approved by the institutional Ethical Review Board at the MGM Institute of Health Sciences, Navi Mumbai, India (No. of approval: MGMIHS/RES./02/2018-19/63). Healthy patients, with or without tobacco habits, were selected from among the individuals who visited the dental hospital for periodontal, orthodontic or surgical treatment, such as crown lengthening, ridge augmentation, pericoronitis, or periodontal flap surgery. After obtaining informed consent from the participants, the discarded NAOM samples taken from the abovementioned surgical procedures were collected. A total of 100 tissue samples were obtained from non-tobacco users (n = 50) and tobacco users (n = 50) for the present study.

Histopathological assessment

All tissue samples were fixed in 10% neutral buffered formalin, followed by tissue processing and staining with the use of hematoxylin and eosin (H&E). The histopathological assessment of the samples was carried out by 2 independent oral pathologists for the presence of microscopic changes, such as basilar hyperplasia and inflammation. Inflammation in the samples was scored as follows: 0 – no inflammation; 1 – mild (less than 25 inflammatory cells); 2 – moderate (more than 25 and less than 125 inflammatory cells); and 3 – severe (more than 125 inflammatory cells).[13]

Immunohistochemical staining

From each paraffin-embedded block, tissue sections were cut to a thickness of 3 µm with a rotary microtome (Leica RM 2245; Leica Camera, Wetzlar, Germany). The sections were dewaxed in xylene, and then rehydrated in absolute alcohol, 95% alcohol and 85% alcohol. Antigen retrieval was performed by immersion in Tris-EDTA (0.1 M, pH 9) in a decloaking chamber at 125°C for 10 min. Endogenous peroxidase activity was blocked by using 3% hydrogen peroxide in methanol for 30 min. The slides were then incubated with an anti-SOX2 rabbit monoclonal antibody (prediluted, Clone EP103, Cat. No. PR071; PathnSitu Biotechnologies, Livermore, USA), an anti-OCT4 rabbit monoclonal antibody (prediluted, Clone EP143, Cat. No. BSB2029; Bio SB, Santa Barbara, USA) and an anti-Nanog mouse monoclonal antibody (prediluted, Clone 5A10; Novus Biologicals, Centennial, USA) diluted in Tris-buffered saline (TBS) and 5% bovine serum albumin (BSA) at 4°C overnight in a wet chamber. For the substrate chromogenic reaction, the sections were immersed in a freshly prepared solution of 0.03% diaminobenzidine for 2 min at room temperature, followed by

counterstaining with Mayer's hematoxylin. The samples of colon carcinoma, esophageal carcinoma and seminoma were used as positive controls, whereas the substitution of the primary antibody with TBS served as an internal negative control for each batch of staining.

Immunohistochemical analysis and scoring

Two pathologists independently reviewed the stained slides without any access to clinical or demographic data of the patients. The nuclear expression of SOX2, OCT4 and Nanog was observed in the epithelial cells of both tobacco users and non-tobacco users. A total of 1,000 cells were counted in a ×400 magnification field per specimen, using the ImageJ software (National Institutes of Health (NIH), Bethesda, USA; https://imagej.nih.gov/ij/). The percentage positivity of all markers was calculated by dividing the total number of positively stained cells by the total number of cells in the section (minimum 1,000 cells) in high-power fields ×100%. Staining intensity was observed and graded as weak, moderate or strong. The scoring of percentage positivity (<5% of cells – 0; 5–24% – 1; 25–49% – 2; 50–74% – 3; and >75% of cells – 4) and intensity (no cells – 0; weak – 1; moderate – 2; and strong – 3) was done according to the methodology proposed by Vijayakumar et al.[14] The final scoring of each section was calculated by adding the scores of percentage positivity and intensity (0–3 – low expression; and 4–7 – high expression). If any disagreement occurred (intensity score discrepancy >1), the slides were re-evaluated by a third independent pathologist along with previous observers to obtain a consensus diagnosis.[9]

Statistical analysis

The observations were noted and recorded in a Microsoft® Excel sheet (Microsoft Corporation, Redmond, USA). The results were compared for statistical significance using IBM SPSS Statistics for Windows, v. 24.0 (IBM Corp., Armonk, USA). Student's independent *t* tests were used to analyze the intra- and intergroup variations in SOX2, OCT4 and Nanog expression. Linear regression analysis was applied to explore independent predictor variables influencing the expression of stem cell markers.

Results

The study participants constituted 2 groups: group I comprised healthy controls with NOM, without any tobacco habit (n = 50); and group II consisted of individuals with NAOM with a tobacco habit (n = 50). The association of the expression patterns of the stem cell markers with the demographic data of both groups are depicted in Tables 1–4.

Expression patterns of SOX2, OCT4 and Nanog, and their correlation with clinicopathological factors in non-tobacco users (group I)

The nuclear expression of SOX2 and OCT4 was observed only in the parabasal layer of NOM, with mild to moderate intensity of immunoreactivity observed for both markers. Nanog was found to be negative for all NOM samples (Fig. 1). The association between the percentage expression of SOX2 and OCT4 and the clinicopathological features of this group, including age, gender and the grade of inflammation, was examined with the independent *t* test. The percentage expression of SOX2 and OCT4 was statistically significantly different among the grades of inflammation ($p < 0.001$), whereas age and gender did not seem to influence their expression (Table 1). Linear regression analysis showed the grade of inflammation to be a significant and independent predictor of expression of these 2 stem cell markers (Table 2).

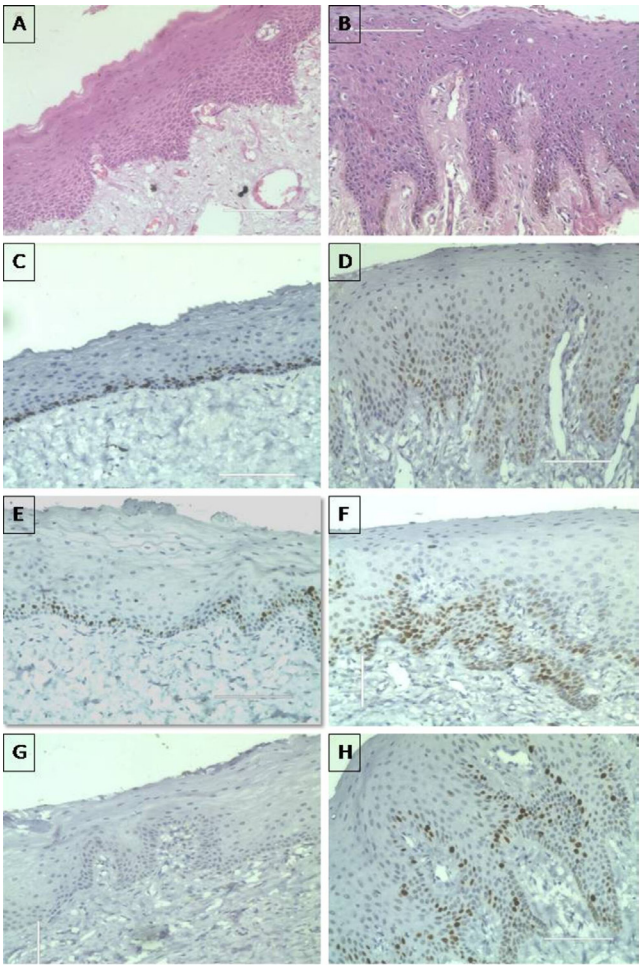

Fig. 1. Histopathological staining of oral mucosa (hematoxylin and eosin (H&E); ×400 magnification) in non-tobacco users (A) and tobacco users (B). In non-tobacco users, the immunohistochemical expression of SOX2 (C), OCT4 (E) and Nanong (G) were found to be limited to the parabasal layer (if positive for staining), whereas the immunoreactivity of all these markers increased up to the basal and suprabasal layers and above in tobacco users (D, F and H for SOX2, OCT4 and Nanong, respectively)

Table 1. Correlation between demographic features and the percentage expression of SOX2 and OCT4 in normal oral mucosa (NOM) in non-tobacco users

Variables		Sample size (n = 50)	Percentage expression of SOX2 [%]	p-value	Percentage expression of OCT4 [%]	p-value
Age [years]	≤40	28	3.12 ±1.59	0.842	0.93 ±0.93	0.853
	>40	22	3.03 ±1.72		0.98 ±1.07	
Gender	male	38	2.83 ±1.64	0.053	0.92 ±0.98	0.723
	female	12	3.88 ±1.40		1.04 ±1.07	
Grade of inflammation	no	34	2.58 ±1.43	<0.001*	0.65 ±0.83	<0.001*
	mild	8	3.21 ±1.56		0.93 ±1.03	
	moderate	8	5.06 ±0.93		2.62 ±0.41	

Data presented as mean ± standard deviation ($M \pm SD$). * statistically significant (unpaired two-sample t test).

Table 2. Multiple regression analysis showing the association of predictive variables with the percentage expression of SOX2 and OCT4 in the normal oral mucosa (NOM) of non-tobacco users

Model		Unstandardized coefficients		Standardized coefficient β	t	p-value	95% CI for β	
		β	SE				lower bound	upper bound
SOX2 expression	(constant)	2.002	0.843	–	2.374	0.022	0.303	3.700
	age	–0.248	0.401	–0.076	–0.618	0.539	–1.055	0.559
	gender	0.622	0.485	0.164	1.284	0.206	–0.354	1.599
	grade of inflammation	0.890	0.331	0.415	2.692	0.010*	0.224	1.556
OCT4 expression	(constant)	0.710	0.527	–	1.347	0.185	–0.352	1.772
	age	0.000	0.242	0.000	0.001	0.999	–0.487	0.488
	gender	–0.286	0.294	–0.125	–0.975	0.335	–0.878	0.305
	grade of inflammation	0.668	0.190	0.513	3.522	0.001*	0.286	1.050

SE – standard error; CI – confidence interval; * statistically significant.

Expression patterns of SOX2, OCT4 and Nanog, and their correlation with clinicopathological factors in tobacco users (group II)

In NAOM, the nuclear expression of all 3 markers was noticed in both basal and suprabasal layers of oral mucosa. The immunoexpression of these markers was analyzed along with the clinicopathological parameters of tobacco users, including age, gender, type of habit (smoking, smokeless and mixed, i.e., with alcohol), duration of habit, and tobacco contact time, and histopathological features, such as basilar hyperplasia and the grade of inflammation. Male gender, mixed habit and the presence of basilar hyperplasia significantly contributed to the alteration of the percentage expression of SOX2, OCT4 and Nanog (Table 3). For SOX2 expression, the type of tobacco habit was found to be a significant predictive variable, whereas age, duration of habit, basilar hyperplasia, and the grade of inflammation emerged as independent predictive variables for OCT4 expression in linear regression analysis. Gender, tobacco contact time and the grade of inflammation were observed to have significantly influenced Nanog immunoexpression (Table 4). The intensity of immunoreactivity of all markers was noticed to increase along with an increase in the grade of inflammation.

Intergroup comparison of SOX2, OCT4 and Nanog expression

The mean value of percentage expression of OCT4 (9.74 ±4.79%) was found to be statistically higher in tobacco users when compared to non-tobacco users (0.95 ±0.99) ($p < 0.001$). A similar increasing trend was observed in SOX2 expression under the influence of tobacco, though the result was not statistically significant. In the case of Nanog, the 2 groups were not compared, as this marker was expressed only in tobacco users and was absent in the NOM of non-tobacco users.

Co-expression of SOX2, OCT4 and Nanog

The co-expression of SOX2, OCT4 and Nanog was assessed by evaluating the percentage expression in the same area of the tissue specimens. In tobacco users, a higher co-expression of SOX2 and OCT4 (76%) was observed in comparison with non-tobacco users (30%), though the statistical correlation was found to be non-significant (Spearman' coefficient $\rho = 0.120$; $p = 0.408$). The co-expression of all 3 markers was observed in 58% of all tobacco users. The overall intensity of immunoreactivity of all markers was observed to be higher in tobacco users in comparison with non-tobacco users.

Table 3. Correlation between demographic features and the percentage expression of SOX2, OCT4 and Nanog in normal-appearing oral mucosa (NAOM) in tobacco users

Variables		Sample size (n = 50)	Percentage expression of SOX2 [%]	p-value	Percentage expression of OCT4 [%]	p-value	Percentage expression of Nanog [%]	p-value
Age [years]	≤40	28	5.06 ±6.20	0.559	9.85 ±7.14	0.908	3.96 ±4.65	0.291
	>40	22	4.13 ±4.62		9.60 ±8.07		2.54 ±4.66	
Gender	male	41	5.38 ±5.82	<0.001*	10.71 ±7.50	0.034*	4.07 ±4.89	0.016*
	female	9	1.37 ±1.37		5.32 ±5.93		0.00 ±0.00	
Type of habit	smoking	15	3.11 ±1.56	<0.001**	12.66 ±7.11	<0.001**	3.71 ±3.58	0.003**
	smokeless	23	2.81 ±4.66		4.54 ±4.04		1.24 ±1.71	
	mixed	12	10.10 ±6.98		15.38 ±6.90		6.72 ±7.21	
Duration of habit [years]	≤5	26	5.94 ±6.20	0.196	12.09 ±7.52	0.060	4.46 ±5.39	0.136
	6–10	16	3.72 ±5.22		7.69 ±6.55		2.71 ±3.90	
	>10	8	2.32 ±1.72		6.22 ±7.35		0.90 ±1.76	
Tobacco contact time [h]	≤1	36	3.84 ±4.37	0.197	9.44 ±7.63	0.662	3.54 ±5.41	0.609
	>1	14	6.73 ±7.57		10.49 ±7.33		2.78 ±1.63	
Basilar hyperplasia	absent	20	1.98 ±1.85	0.001*	5.44 ±5.80	<0.001*	1.45 ±1.72	0.016*
	present	30	6.44 ±6.42		12.60 ±7.17		4.59 ±5.55	
Grade of inflammation	no	9	3.33 ±3.09	0.535	5.96 ±8.01	0.025*	1.91 ±1.92	0.260
	mild	15	4.73 ±6.52		6.09 ±6.11		1.98 ±1.77	
	moderate	24	4.68 ±4.96		12.37 ±7.05		4.63 ±6.27	
	severe	2	9.80 ±13.85		16.30 ±8.02		4.25 ±0.63	

Data presented as mean ± standard deviation (*M* ±*SD*). * statistically significant (unpaired two-sample *t* test); ** statistically significant (one-way ANOVA (two-tailed)).

Table 4. Multiple regression analysis showing the association of predictive variables with the percentage expression of SOX2, OCT4 and Nanog in the normal-appearing oral mucosa (NAOM) of tobacco users

Model		Unstandardized coefficients		Standardized coefficient	t	p-value	95% *CI* for β	
		β	*SE*	β			lower bound	upper bound
SOX2 expression	age	−0.636	1.886	−0.058	−0.337	0.738	−4.448	3.176
	gender	−2.913	2.903	−0.204	−1.004	0.322	−8.781	2.954
	type of habit	3.259	1.254	0.436	2.599	0.013*	0.724	5.793
	duration of habit	0.701	1.451	0.095	0.483	0.632	−2.232	3.633
	tobacco contact time	−0.972	2.078	−0.080	−0.468	0.642	−5.171	3.227
	basilar hyperplasia	2.075	1.710	0.186	1.214	0.232	−1.381	5.531
	grade of inflammation	0.343	1.090	0.052	0.315	0.754	−1.860	2.547
OCT4 expression	age	4.971	2.131	0.333	2.332	0.025*	0.663	9.278
	gender	−4.440	3.302	−0.230	−1.347	0.185	−11.120	2.225
	type of habit	0.552	1.644	0.055	0.336	0.739	−2.771	3.875
	duration of habit	−4.020	1.634	−0.403	−2.463	0.018*	−7.329	−0.722
	tobacco contact time	−5.060	2.676	−0.307	−1.892	0.066	−10.470	0.346
	basilar hyperplasia	4.460	1.937	0.295	2.304	0.027*	0.547	8.378
	grade of inflammation	4.302	1.223	0.477	3.517	0.001*	1.830	6.773
Nanog expression	age	0.694	1.464	0.075	0.474	0.638	−2.265	3.653
	gender	−4.752	2.049	−0.395	−2.320	0.026*	−8.893	−0.612
	type of habit	1.739	1.028	0.276	1.691	0.099	−0.340	3.817
	duration of habit	−0.904	1.125	−0.145	−0.804	0.426	−3.177	1.369
	tobacco contact time	−5.407	1.590	−0.526	−3.400	0.002*	−8.621	−2.193
	basilar hyperplasia	1.089	1.322	0.115	0.824	0.415	−1.582	3.760
	grade of inflammation	2.132	0.839	0.380	2.540	0.015*	0.436	3.829

* statistically significant.

Discussion

The cumulative evidence of a positive correlation between the immunoexpression of SOX2, OCT4 and Nanog and various oral potentially malignant disorders and oral carcinoma indicates the putative role of these proteins in oral carcinogenesis. Though previous research has reported on the presence or absence of these pluripotent stem cell markers in normal mucosa as compared to that of the lesional tissues, an exclusive study targeting their expression in adult stem cells in NOM has not been conducted until now. In this context, the present study observed only SOX2 and OCT4 expression in NOM, which is in agreement with the observations of Qiao et al.,[10] whereas all the samples showed negative immunoreactivity toward Nanog.[10,15] SOX2 is thought to be a guardian of the embryological development of the head and neck region through its expression in neural crest cells. Since most of the oral and orofacial structures are derived from the migrated neural crest cells, the presence of SOX2 in the NOM of non-tobacco users explains its putative role in oral mucosa differentiation through a precise cell flow.[16]

The lower mean value of OCT4 percentage expression as well as the absence of Nanog expression in NOM could be explained by the regulatory and repressing effect of SOX2 on OCT4 and Nanog during specific lineage differentiation.[11,17] We observed the parabasal layer expression of these pluripotent stem cells, in contrast to the basal layer expression reported by many authors. As NOM is thought to have its stem cell population distributed in the quiescent basal cell and active parabasal cell layers, this justifies the peculiar pattern of parabasal layer expression of SOX2 and OCT4 rather than the basal cell layer expression, as observed in previous studies.[18] The presence of inflammation not only was found to be significantly associated with an increased immunoreactivity of these markers, but also emerged as a predictive variable for the same in the NOM of non-tobacco users. Inflammation has an inducible effect on stem cell proliferation through cytokine production. In NOM, even sterile inflammation (non-microbial), which may result from chemical and physical insults, can cause the proliferation of stem cells. At the site of injury, inflammatory cells recognize danger-associated molecular patterns and secrete molecules that prime the tissue restoration via stem cell induction.[19]

In tobacco users, an increased expression of SOX2 and OCT4 and the appearance of Nanog in tobacco-affected oral mucosa were observed. Naini et al. reported similar results in the adjacent non-tumor oral tissue, which indicated the potential role of these markers in the early molecular stages of carcinogenesis.[12] Fu et al. also observed a similar higher expression of Nanog in the corresponding tumor-adjacent normal tissues as compared to their normal counterparts, which supports its underexplored behavior in carcinogenesis.[9] Significant associations between tobacco-related parameters, including the type of habit and tobacco contact time, and an increased immunoreactivity of all these markers indicate a critical role of nicotine in oral carcinogenesis through the regulation of the expression and stemness of SOX2, OCT4 and Nanog.[20] In addition, a mixed habit that includes alcohol use was shown to be associated with an increased expression of all of these ES cells markers, which is in accordance with the experimental findings showing the alcohol-activated induction of cancer stem cells.[21]

Histopathological factors, including basilar hyperplasia, were correlated with an elevated expression of stem cell markers in the present study. Under the influence of tobacco, the shift and expansion of the stem cell niche from the parabasal cell layer (active stem cell population) to the basal cell layer (quiescent stem cell population) was evident from the basal cell hyperplasia observed in histopathological examinations and the criteria depicting architectural changes in epithelial dysplasia. In addition, the grade of inflammation also correlated positively with an increased expression of stem cell markers. Both smokeless and smoke tobacco can cause genetic and proteomic alterations through the dysregulation of inflammation-associated pathways, such as MAPK/ERK as well interferon signaling in oral keratinocytes.[22]

The mean percentage expression values of all of the markers were significantly elevated in tobacco users in comparison with non-tobacco users, which may indicate the cumulative effect of long-term tobacco exposure on oral keratinocytes by inducing the expansion of the embryonic stem cell population. The co-expression of SOX2 and OCT4 was found to be increased in the oral mucosa of tobacco users, similar to the observations made by Qiao et al., who reported an elevated $SOX2^{+}OCT4^{+}$ profile in the transforming oral mucosa of the rat and in precancerous lesions of humans that displayed simple hyperplasia.[10] The co-expression of all 3 markers in over a half of tobacco users could be a result of the mechanical and chemical impact of tobacco on oral keratinocytes.

Conclusions

The use of tobacco and the tobacco habit can induce early molecular changes at the cellular level through the expansion of the stem cell niche, which could be recognized histopathologically as basilar hyperplasia. Increased expression and co-expression of pluripotent stem cell markers SOX2, OCT4 and Nanog in NAOM may indicate another molecular pathway of tobacco-induced oral carcinogenesis. The observations of the present study could be used as a baseline for further research on the impact of tobacco on oral mucosal stem cells in the development of various oral potentially malignant disorders and oral cancer.

Ethics approval and consent to participate

The research protocol was reviewed and ethically approved by the institutional Ethical Review Board at the MGM Institute of Health Sciences, Navi Mumbai, India (No. of approval: MGMIHS/RES./02/2018-19/63). Informed consent was obtained from all participants.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Effect of the application of software on the volumetric and cross-sectional assessment of the oropharyngeal airway of patients with and without an open bite: A CBCT study

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Abstract

Background. Using different software to evaluate the airways, with different thresholds, but within the range for airway recognition, could yield different measurements in the same patient with or without craniofacial disharmony.

Objectives. The aim of the present study was to compare the volume and the most constricted area (MCA) of the oropharynx in individuals with or without an open bite by using 2 software programs meant for cone-beam computed tomography (CBCT).

Material and methods. This comparative study included 60 cases selected from 137 CBCT scans obtained from individuals with the presence or absence of an open bite. Each group included adults of both genders – in total 30 women and 30 men – with a mean age of 27.57 ±11.85 years in the open bite group and 26.23 ±6.78 years in the control group. The oropharyngeal volume and MCA were measured with 2 three-dimensional (3D) software packages: Planmeca Romexis®; and Nemotec NemoStudio®. Two calibrated orthodontists trained in the use of the software made the measurements. Data was analyzed using Student's t tests for independent and paired samples ($p < 0.05$).

Results. In general, the oropharynx volume measurements obtained with the NemoStudio software were significantly higher than those obtained with Romexis (19,007.17 ±8005.79 mm^3 and 17,823.47 ±7148.62 mm^3, respectively) ($p = 0.020$). However, when the groups were analyzed separately, the measurements of the group with an open bite did not differ according to the software used ($p = 0.352$). The measurements of the MCA of the oropharynx were significantly higher when obtained with the NemoStudio software (MD (mean difference) = 19.02 mm^2) ($p = 0.005$). In contrast, no difference in the MCA results for the 2 software packages was found in the open bite group ($p = 0.728$).

Conclusions. The volumetric and cross-sectional measurements of the oropharyngeal airway, particularly in individuals without an open bite, were affected by the software used.

Keywords: airways, software, cone-beam computed tomography, CBCT, open bite

Introduction

Craniofacial disharmony is considered a predisposing factor for respiratory disorders during sleep in children.[1] The symptoms of respiratory disorders are reportedly associated with facial and dental morphometry,[2] highlighting the important role of craniofacial and airway morphology.[3] Hence, an open bite accompanied by clockwise mandibular rotation and different associated factors, including the skeletal pattern, influence the airway dimensions. The repositioning of the mandible significantly affects these dimensions.[4]

In context of the above, conducting airway studies is vital. The research was initially performed using lateral radiographs. However, with the emergence and development of new technologies, computed tomography (CT) is currently being applied.[5,6] The measurements based on two-dimensional (2D) images have been questioned, taking into account the three-dimensional (3D) structures of the airways. However, studies have found a similarity between the linear measurements obtained with lateral radiographs and those obtained with cone-beam computed tomography (CBCT),[7] as well as a correlation between the sagittal and axial areas and the volume on 2D images.[8] In the search for more reliable information about the airways, recent research findings have validated the use of both lateral radiographs and CBCT.[9,10]

With technological advancement, different software packages for airway analysis have been developed. A systematic review conducted in 2011 reported 18 software packages for airway studies.[5] However, this review did not mention the threshold (automatic or interactive) or the measurement method (automatic or manual) used,[5] which only a few studies specified. Indeed, only one study determined the ideal threshold for the airway volume under experimental conditions[11]; therefore, this threshold cannot be used as a standard protocol for airway assessment. Another study used a fixed threshold, which was based on the average of previously determined thresholds for each tomography scan.[12] The objective of that study was to delimit the airways by using reliable references based on the 3D images obtained with the use of the Dolphin™ software in order to establish a protocol and to set normative upper airway values for patients with the following characteristics: Caucasian; adult (23–35 years); healthy; Class I occlusion; and a Class I profile without asymmetries.[12] In terms of the measurement method used, studies have examined the use of different software packages and found high reliability for automatic measurements, which differed from manual measurements.[13] Software packages are continually updated and improved, prompting the question of whether the above issues have been corrected. Several studies have used prototypes or phantoms to verify the obtained results.[6,11,14,15] However, if such devices are to be used to represent the airways, the materials they are made of should have the same density as soft tissues to ensure that the threshold values obtained with these prototypes are representative.[16] Furthermore, using different tomography modalities to scan the same tissue can lead to significant differences in the results,[17] and differences in the voxel and artifact sizes can affect CBCT images.[18,19] Recently, a study comparing 2 software packages, Invivo™ and Dolphin, was conducted, using fixed and interactive thresholds, respectively. It aimed to propose normative values for children to be used in the diagnosis and early management of pediatric sleep apnea, and they found differences depending on which software was used.[20] The same software packages were applied to make measurements in adults and in prototypes used as a control in another recent study measuring the volume, the minimum area and the location of the minimum area.[21] The authors of that study also found overestimated values when Dolphin was used and underestimated values when Invivo was used, though both software packages were considered reliable and strongly correlated.[21]

The aforementioned issues raised the question of whether different software packages used with different thresholds, but within the range for airway recognition, would yield different measurements in a sample of patients with craniofacial disharmony, in whom the dimensions of the airways may be altered. Therefore, the objective of the present study was to determine the volume and the most constricted area (MCA) of the oropharynx in individuals with and without an open bite and with different skeletal patterns by using the automatic software mode for Romexis® (Planmeca, Helsinki, Finland) and NemoStudio® (Nemotec, Madrid, Spain) in 3D, applying different thresholds.

Methodology

This retrospective and cross-sectional study was approved by the Research Committee and Ethics Committee of the Scientific University of the South (Universidad Científica del Sur – UCSUR), Lima, Peru, and Federal University of Rio Grande do Sul (Universidade Federal do Rio Grande do Sul – UFRGS), Porto Alegre, Brazil (No. of approval: 2018-00014). The sample for this study included 60 cases selected from 137 CBCT scans obtained from a private imaging center in Lima, Peru. The CBCT scans were done for reasons other than the present study. Gender and the skeletal pattern were considered inclusion criteria. Based on different skeletal patterns, both anteroposterior and vertical, the sample was divided into 2 groups: 30 CBCT images from individuals with an open bite; and 30 CBCT images from individuals without an open bite (the control group). To describe the sagittal and vertical features of both groups, we evaluated the skeletal relationship (A point–nasion–B point angle (ANB)), facial divergence (Frankfort mandibular plane angle (FMA)) and the vertical overbite depth indicator (ODI), as shown in Table 1.

For this purpose, cephalograms were derived from the CBCT scans. To get an adequate distribution of the sample related to the anteroposterior skeletal patterns, Class I (ANB = 2 ±2°), Class II (ANB > 4°) and Class III (ANB < 0°) were included in both groups, and the following characteristics of overbite were considered: the open bite group – 0 mm or negative; and the non-open bite group – positive. The CBCT images were taken from 30 males and 30 females, with 15 males and 15 females in each group, with or without an open bite, and aged between 15 and 56 years. The mean age was 27.57 ±11.85 years for the open bite group and 26.23 ±6.78 years for the non-open bite group. All excess CBCT images were excluded after the required number of 60 was achieved. The sample was obtained as scrutinized in the flow chart (Fig. 1).

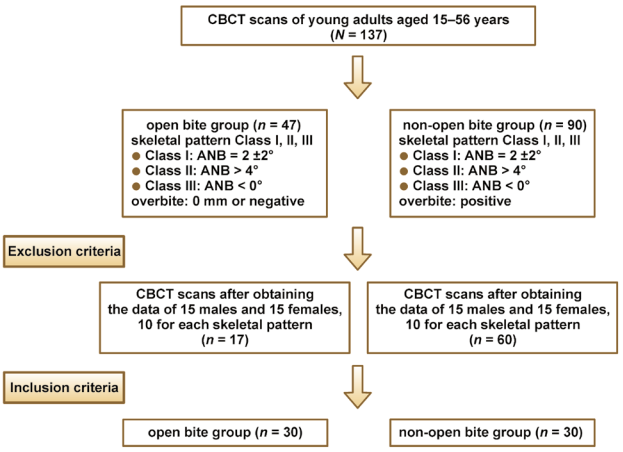

Fig. 1. Flow chart of the sample collection
CBCT – cone-beam computed tomography.

Image acquisition

Cone-beam computed tomography was performed according to the standardized protocol of the radiological center and the images were obtained by means of the Picasso Master 3D scanner/E-woo model (Vatech, Hwaseong, South Korea). The CBCT scans were acquired with the patient in a seated and erect position, and with a craniocervical alignment of 90–110°. The sagittal midline, the Frankfort plane, the occlusal plane, and the incisor line were the anatomical points used to position the head. Instructions were given to the patients to keep their eyes open, not to move their head, not to swallow, to breath slowly, and to be in maximum intercuspation during scanning. The following settings of the 3D scanner were used to acquire the images: 8 mA; 90 kVp; flat-panel detector 25 × 20 cm^2 with a field of view (FoV) of 20 × 19 cm^2; an isotropic voxel size of 0.3 mm; and an exposure time of 20 s.

Table 1. Initial characteristics of the samples in both groups

Measurement	Group	*n*	*M* ±*SD*	*p*-value
SNA [°]	non-open bite	30	85.30 ±4.48	0.112
	open bite	30	83.64 ±3.41	
SNB [°]	non-open bite	30	82.90 ±5.88	0.571
	open bite	30	82.02 ±6.08	
ANB [°]	non-open bite	30	2.41 ±3.81	0.501
	open bite	30	1.63 ±5.01	
FMA [°]	non-open bite	30	29.27 ±5.76	0.244
	open bite	30	31.05 ±5.98	
ODI [°]	non-open bite	30	61.70 ±10.36	0.042*
	open bite	30	56.65 ±8.31	
Age [years]	non-open bite	30	26.23 ±6.78	0.595
	open bite	30	27.57 ±11.85	

SNA – sella–nasion–A point angle; SNB – sella–nasion–B point angle; ANB – A point–nasion–B point angle; FMA – Frankfort mandibular plane angle; ODI – vertical overbite depth indicator (ODI); *M* – mean; *SD* – standard deviation; * statistically significant (independent *t* test).

The CBCT images were converted into the DICOM (Digital Imaging and Communications in Medicine) format, and analyzed with the Planmeca Romexis software, v. 5.1.1.R, and the Nemotec NemoStudio software, v. 2017 (NemoFAB).

Calibration

In order to ensure uniform intra- and inter-observer measuring procedures, a calibration process was performed. Ten CBCT scans were randomly selected from the sample and the variables of interest were measured twice at an interval of 15 days between the 2 measurements for both software packages studied.

Measurement of variables

A Toshiba Satellite L845, Intel Core i3, 8 GB RAM computer, with a 64-bit operating system (Toshiba, Tokyo, Japan), was used for the selected upper airway measurements. The orientation of the head in the reconstruction of all 3D images was standardized by aligning the Frankfort plane (porion–orbitale (Po–Orb) on both sides) with the horizontal guideline in the sagittal and coronal sections. The anterior nasal spine (ANS) and the posterior nasal spine (PNS) were aligned with the vertical guideline, and the pupils were aligned with the horizontal guideline. Both alignment procedures were performed in the axial section.

For some years, the automatic airway measurement method has been used by most, if not all, software packages. The Romexis and NemoStudio software require a manual procedure to establish the limits of the selected part of the airway being measured. Indeed, these software packages, when used for the detection of the airway at the level of the oropharynx, define limits by prism, cube, or by locating points along the airway, including the location of the seed point (s-point), and the established threshold or tolerance.

For the measurements of volume and MCA in Romexis, the head icon was used to perform the measurements in an automatic mode. The procedure for demarcating the airway limits involved locating the vertical guide as central as possible in the oropharynx and perpendicular to the horizontal guide. Next, one point was located at the intersection of the vertical guide with the upper limit of the oropharynx, which begins in PNS, and then runs posteriorly toward the vertebrae. The horizontal guide was located at the lower limit of the oropharynx, tangent to vertebra C3 in its most caudal medial portion, which is directed forward. The 2nd point was placed at the lower limit of the oropharynx, following the projection of the 1st point at the intersection with the vertical guide. With this 2nd point, the manual procedure to demarcate the airway limits was completed by clicking 'done'. The anterior limit of the oropharynx was automatically defined by the formation of a cube where the anterior vertical line starts from PNS and extends toward the lower limit, and where the posterior limit of the oropharynx is defined by a line parallel to the anterior limit located on the vertebrae. The lateral walls of the pharynx and the total extensions of the lateral projections were included automatically. The measurement of the airway was done automatically. In order to standardize the protocol, a threshold of 300 HU (Hounsfield unit) was used for all of the images. The value was chosen while establishing the protocol as with this threshold, the researcher could visualize all images of the upper airways clearly (Fig. 2).

In NemoStudio, the NemoFAB component containing a tool for measuring the airways was used. In this program, the procedure was performed by demarcating the upper limit of the oropharynx. Starting at PNS, the procedure continued in a posterior direction along the horizontal guide until the middle of the vertebra was visible at that height, and then descended to the lower limit of the oropharynx, passing the horizontal guide line, tangent to the middle caudal portion of C3. From this point, the procedure was directed forward until a point that was in the direction of PNS was reached. From that point, and in the direction of the upper limit of the oropharynx, PNS

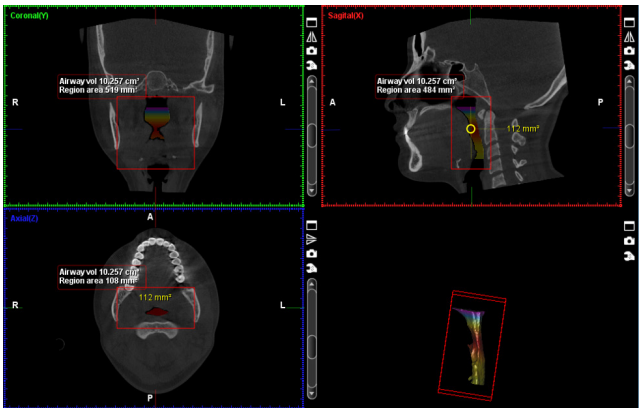

Fig. 2. Evaluation with the use of the Romexis software – upper airways, oropharynx, volume, and the most constricted area (MCA)

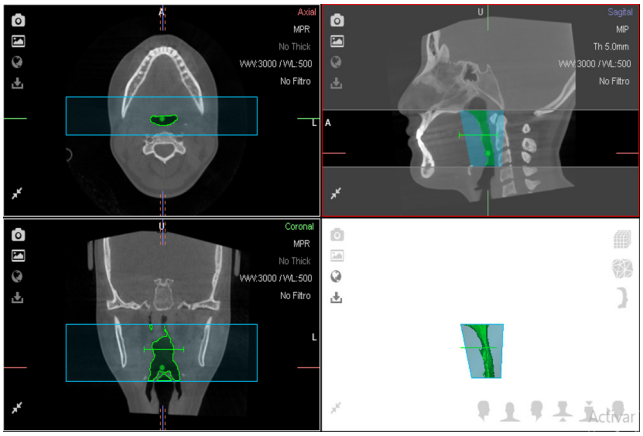

Fig. 3. Evaluation with the use of the NemoStudio software – upper airways, oropharynx, volume, and the most constricted area (MCA)

was reached again, forming a prism. Next, an s-point was placed on the epiglottis within the demarcated area and the measurement of the airway was done automatically. The tolerance was defined as 500 HU (Fig. 3).

Statistical analysis

For this study, the statistical program IBM SPSS Statistics for Windows, v. 22.0 (IBM, Inc., Armonk, USA), was used. The normality of the distribution of quantitative variables was assessed with the Shapiro–Wilk test. Thereafter, the sample characteristics were evaluated by applying Student's independent-samples *t* test. To compare the mean values of the volume and the MCA of the oropharynx determined by the different software packages for both the total sample and each group, Student's paired-samples *t* test was used for related samples. The statistical tests were performed at a significance level of $p < 0.05$. A calibration process was performed previously by applying the intraclass correlation coefficient (*ICC*) for both software packages.

Results

The results from the *ICC* analysis showed intra-observer values of 0.999 and 0.994 for volume and MCA, respectively, and inter-observer values of 0.982 and 0.888 for volume and MCA, respectively, for Romexis. NemoStudio showed intra-observer values of 0.994 and 0.995 for volume and MCA, respectively, and an inter-observer value of 0.997 for both variables.

Table 1 displays the characteristics of both groups, which tend to show maxillary protrusion and a well-positioned but slightly protruding mandible in the group without an open bite. The ANB showed skeletal pattern variability in both directions, which was slightly more marked in the open bite group. The FMA showed that both groups were hyperdivergent, and the mean age indicated that the sample consisted of young adults. The only variable that pre-

sented a significant difference was ODI. In this regard, the open bite group presented more marked values of a skeletal open bite as compared with the group without an open bite, as well as skeletal open bite characteristics, despite a lack of clinical expression of an open bite.

Table 2 compares the measurements of the volume and the cross-sectional assessment of the MCA of the oropharynx performed with the Romexis and NemoStudio software. The table shows significant differences in the average volume for the entire sample between the two software packages (Romexis: 17,823.47 ±7,148.62 mm^3; NemoStudio: 19,007.17 ±8,005.79 mm^3) (p = 0.020). The average values of MCA for the entire sample also showed significant differences between the 2 software packages (Romexis: 227.32 ±102.61 mm^2; NemoStudio: 246.34 ±118.97 mm^2) (p = 0.005). For both the volume and MCA, higher values were observed with NemoStudio.

Table 3 compares the volume and the MCA of the oropharynx obtained with both software packages for the group with an open bite and the group without an open bite. A significant difference was observed between the 2 software packages in terms of volume for the group without an open bite (Romexis: 17,051.90 ±7,193.83 mm^3; NemoStudio: 18,747.00 ±8,496.91 mm^3) (p = 0.021). The group with an open bite did not show a significant difference in volume (Romexis: 18,595.03 ±7,140.37 mm^3; NemoStudio: 19,267.33 ±7,619.58 mm^3) (p = 0.352). However, the volume values for this group were higher with both software packages than the values obtained for the group without an open bite. The difference in MCA determined by the 2 software packages was more noticeable in the group without an open bite (Romexis: 216.43 ±111.78 mm^2; NemoStudio: 251.68 ±130.06 mm^2) (p = 0.001), while in the open bite group, the MCA values were 238.20 ±93.17 mm^2 with Romexis and 240.99 ±108.73 mm^2 with NemoStudio (p = 0.728).

Discussion

Despite the time elapsed and the efforts made, no gold standard is available for use as a reference for airway measurements,[6] even though airway problems are relatively frequent, with a prevalence of snoring of approx. 14.51%.[22] The literature on airway analysis highlights the need to address several factors with more studies. A thorough examination of the airways that includes information only on the volume would not help to identify the areas of greatest constriction.[23,24] Therefore, the development of software packages that would allow reliable, valid and efficient airway measurements, as well as the comparisons of the semiautomatic and manual measurement methods,[25] are still issues of concern for researchers.

Upper airway changes are observed over adolescence and late adolescence due to the periods of growth.[26–28] At maturity, the likelihood of changes is lower, and for that reason, the analysis of an adult sample enables the accurate comparisons of the groups.[29] Also, studies performed to determine the accuracy of the measurements of oropharyngeal morphology indicated that the images obtained with the Vatech equipment yielded more precise measurements of the volume and the axial area.[6]

Table 2. Comparison of the oropharyngeal volume and most constricted area (MCA) between the 2 evaluated software packages with regard to the total sample

Measurement	*N*	*M* ±*SD*	*p*-value	*MD*	95% *CI*	
					lower limit	upper limit
Volume with RS [mm^3]	60	17,823.47 ±7,148.62	0.020*	−1,183.70	−2,178.01	−189.39
Volume with NS [mm^3]	60	19,007.17 ±8,005.79				
MCA with RS [mm^2]	60	227.32 ±102.61	0.005*	−19.02	−31.95	−6.09
MCA with NS [mm^2]	60	246.34 ±118.97				

RS – Romexis software; NS – NemoStudio software; *MD* – mean difference; *CI* – confidence interval; * statistically significant (paired-samples *t* test).

Table 3. Comparison of the oropharyngeal volume and most constricted area (MCA) between the 2 evaluated software packages within both groups

Group	Measurement	*N*	*M* ±*SD*	*p*-value	*MD*	95% *CI*	
						lower limit	upper limit
Non-open bite	volume with RS [mm^3]	30	17,051.90 ±7,193.83	0.021*	−1,695.10	−3,113.99	−276.21
	volume with NS [mm^3]	30	18,747.00 ±8,496.91				
	MCA with RS [mm^2]	30	216.43 ±111.78	0.001*	−35.25	−54.50	−16.00
	MCA with NS [mm^2]	30	251.68 ±130.06				
Open bite	volume with RS [mm^3]	30	18,595.03 ±7,140.37	0.352	−672.30	−2,126.25	781.65
	volume with NS [mm^3]	30	19,267.33 ±7,619.58				
	MCA with RS [mm^2]	30	238.20 ±93.17	0.728	−2.79	−19.04	13.46
	MCA with NS [mm^2]	30	240.99 ±108.73				

* statistically significant (paired-samples *t* test).

The knowledge of the software being used is important, and the computer characteristics must be suitable for the software being used. The Romexis tools used for the measurements of the upper airway volume and area, as well as MCA, are easy to learn and apply. The jar icon is designated for manual procedures to demarcate limits by obtaining a cube, and then placing an s-point for the automatic measurement of the airway. Also, a human head icon is available for a simpler manual procedure to demarcate the limits of the airway. By locating at least 2 points, one at the upper limit and one at the lower limit, and then by clicking 'done', it is possible to obtain the cube and the measurement of the airway automatically. Other tools for identifying angle and linear measurements are also available. Obviously, the user must be able to recognize the anatomic structures and to establish a protocol for the measurements.

NemoStudio has an organized protocol established, since upper airway measurements are part of surgical procedures for treatment. NemoFAB is divided into sections and NemoFAB upper airway measurement tools belong to section E. With the Romexis software, it is also possible to verify all airway measurements. In the case of the Romexis software head icon and the NemoFAB software, verification is only possible after the measurements have been obtained. It may translate into the amount of time required to make the measurements and it depends on the researcher's desire to work.

Studies on the airway measurement software have mainly evaluated its accuracy, precision and reliability.[10,14,15] Several of these studies used phantoms or prototypes as the gold standard, which resulted in the underestimation of the dimensions, although the software showed the reliability of the obtained data.[14–16] Although the results were highly correlated, the software did not have good accuracy, which suggests systematic errors.[13] Other studies revealed that although some software packages provided underestimated results while others provided overestimations, they were still considered reliable.[19,20] The present study did not use any gold standard; consequently, the values may be underestimated or overestimated relative to the reference values provided in the literature. It would be desirable for upper airway studies that include the use of software packages to describe the characteristics of the software. Since many of software packages are only commercially available, the information they provide is oriented to selling, and not to the details of the approaches, especially regarding the behavior of algorithms in each software package. That is why it is difficult to find the equivalent value for each approach.

The software packages that are most frequently compared or evaluated are Mimics® and Dolphin (the measurements of the airway volume)[10]; Amira®, 3Diagnosys® and OnDemand3D® (the measurements of the volume, the minimum axial area and the length)[14]; Mimics, ITK-SNAP, OsiriX®, Dolphin 3D, Invivo, and OnDemand3D (the measurements of the volume)[15]; 3dMvultus® (the measurements of the volume, the area and the length)[16]; and Dolphin 3D, Invivo, OnDemand3D, and OrthoSegment® (the measurements of the upper airway volume).[13] One study compared the Beta NemoCeph® 3D and Invivo5 programs with lateral cephalometric radiographs, but only to determine the coincidence of the reference points used in the study.[30] Recently, a study compared Invivo5 and Romexis (v. 3.8.2.R) programs in order to test the reliability of the software in measuring the upper airways.[31] The present study compared the Romexis and NemoStudio software packages for measuring the volume and the MCA of the oropharynx. With Romexis, the head icon was used for a simpler, automatic airway measurement, and the NemoStudio program had the NemoFAB automatic airway measurement tool. Both software packages required a previous manual procedure to establish airway limits, and although it was not manual segmentation, the authors of the study would consider it as a semiautomatic method to obtain airway measurements.

The results of a study comparing 6 programs revealed that segmentation is influenced by the selected threshold, the algorithm of the software and the complexity of the airway.[15] The study also revealed that when an interactive threshold was used, significant differences were found between the volume measurements of the different software packages, and a lower error rate occurred due to the underestimation of the volume as compared to the gold standard. When a fixed threshold was used, the total differences were also significant, but the rate of error among the software packages was greater, even though they yielded the same results for the measurements of the phantom. This phenomenon may be due to differences in the algorithms used in each software package to identify the most complex morphology of the oropharynx.[15] The results of the present study are consistent with investigations using a fixed threshold, since the comparison of the measurements performed with the Romexis and NemoStudio software showed significant differences for the entire sample. However, the threshold used in both software packages was within values that can differentiate bone (250–1,000 HU) from air (between –600 and –1,000 HU).[32] Cone-beam computed tomography is not used in a routine examination, since there are specific indications for it; it offers an advantage over multi-slice computed tomography (MSCT) by generating a lower dose of radiation and being less expensive. The airways can be evaluated with MSCT, also known as multi-detector computed tomography (MDCT), or with CBCT.[6] Usually, Hounsfield units (HU) are used for MDCT, as its characteristics are adequate for mA and kVp. The HU method is used to measure the airways in CBCT. Since the pharyngeal evaluation in CBCT is made through the scale of gray tones, due to a great variability in the sensitivity of different commercially available software packages, different images are acquired, even with the same tomography equipment.

Studies have demonstrated diversity in the values obtained with the airway measurement tool available for the assessment of the pharyngeal space.[7,9] In the present study, when the values obtained from the 2 software packages were compared, no significant differences were found for the open bite group. However, significant differences were found for the group without an open bite. This difference may be related to the sample itself, since the airway dimensions determined by both software packages were smaller for the non-open bite group. Due to the intentional use of a lower threshold in the Romexis software, the values remained lower than those determined with the use of the NemoStudio software, which corresponds to the theory that "the higher the threshold used, the greater the value obtained" and vice versa.[11] Most likely, the configuration of the Romexis software algorithm compensated for using a low threshold and did not show significant differences in the open bite group, since the dimensions of the airways were greater in this group than in the group without an open bite. This was regardless of the software used due to the tendency to maxillary protrusion and non-retrognatic mandible in the individuals from our sample, and a lesser value of ANB in the open bite group. The aforementioned issue is referred to just to provide a better understanding, but the focus of this study was the influence of software, and there is no intention to draw attention to the findings of previous studies.[33–35]

Since the group without an open bite had smaller dimensions according to both software packages, and given the intentional use of a higher threshold for the NemoStudio software, as well as the relationship of this threshold with the algorithm configuration, the values obtained with NemoStudio were higher, leading to significant differences in the measurements for the non-open bite group.

Other studies measuring different structures have suggested that the influence of the threshold on the systematic error in the voxel measurement method of the programs may affect measurements in small structures, but not in large ones.[19] However, this would require a true value to obtain a constant value, which is generally provided by the measurement of a prototype or a phantom as the gold standard; however, this did not occur in the present study. Nonetheless, we might consider the possibility that the threshold could affect the airway measurements due to the complexity of the airways, and would be particularly likely in the group without an open bite, as the airways in that group were smaller. The literature also mentions the effect of partial volume and density due to the voxel size in the software depending on the threshold, where high threshold values would underestimate the measurement, whereas low values would overestimate it by forming a hybrid voxel when it includes 2 structures of different density.[19] In the present study, this theory would not apply, since the Romexis software used a lower threshold than the NemoStudio software. According to the literature, the ideal threshold value for measuring the volume has not been standardized, as the airway volume has been found to vary with regard to the selected threshold,[11,13] and greater or smaller airway volume values result from an increase or a decrease in the threshold, respectively.[11] The present study supports this direct relationship between a higher threshold and a greater volume. Finally, differences between these 2 software packages, including the time used to obtain measurements, also depends on the characteristics of the computer used. Furthermore, using an ideal graphics card, as well as the individual capability of the user factor into these differences. Based on time registration during the establishment of the protocol through the development of videos for manual procedures for automatic airway measurements, it is clear that the time spent on measurements also depends on head positioning and, to some extent, on the selected structures. In the Romexis program, obtaining information with the use of the head icon is faster than using the jar icon. It is comparable to the NemoFAB automatic airway measurement in terms of the time required, since verification can only be done after the measurements have been obtained, and only if an ideal graphics card has been used.

An advantage of the NemoStudio software is that it can delineate the soft palate. Furthermore, the time required to perform airway measurements using the automatic mode varies, depending on the computer characteristics and previous manual procedures. This gives a definitive advantage over the manual mode reported in the literature for other software packages.[24] Additionally, Romexis facilitates the visual identification of MCA through the use of color differentiation to indicate its location and value (Fig. 2), whereas NemoStudio uses solid colors and values that appear only beyond the image (Fig. 3). Both software packages require from the user the adherence to the standardized protocol in addition to the interactive thresholds reported in the literature to ensure a low error rate in the measurement of the structures.[15]

The sample of skeletal and clinical open bite group was not easy to find in CBCT records. From a clinical point of view, special care must be taken in the planning of extraction treatment or orthodontic treatment combined with surgical procedures for patients with an open bite. Indeed, the position of the tongue is usually more anterior in those patients, and treatment plans must be adapted according to the risk of malocclusion risk to the modified position of the soft palate position, commonly affected by treatment. Also, respiratory function therapy must take in account the relationship between the volume and ANB. For diagnosis, and while planning orthodontic or surgical treatment, it is important to pay attention to both groups studied. The non-open bite group had structures with small dimensions and the risk might go unnoticed, whereas in the open bite group, the greater the volume obtained, the lower the chance of finding differences between the software packages studied due to influence of the threshold or the segmentation mode. Finally, other variables related to the modification of the airways, such as obesity and apnea, have not been evaluated. However, their influence could be evaluated in future studies.

Conclusions

The volumetric and cross-sectional assessment of the oropharyngeal airway, particularly in individuals without an open bite, may affect the measurement results, depending on the use of the instruments available for particular software.

Ethics approval and consent to participate

The study was approved by the Research Committee and Ethics Committee of the Scientific University of the South (Universidad Científica del Sur – UCSUR), Lima, Peru, and Federal University of Rio Grande do Sul (Universidade Federal do Rio Grande do Sul – UFRGS), Porto Alegre, Brazil (No. of approval: 2018-00014). Written informed consent was obtained from all participants.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Anatomical localization of posterior superior alveolar artery: A retrospective study by cone-beam computed tomography

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Abstract

Background. Familiarity with the anatomy of the arteries in the sinus wall is essential to prevent the perforation of the sinus membrane and bleeding during dental implant surgery.

Objectives. The aim of the study was to evaluate the anatomical position of the posterior superior alveolar artery (PSAA), using cone-beam computed tomography (CBCT).

Material and methods. A total of 245 CBCT scans met the eligibility criteria for this cross-sectional study. The vertical distance from the lower border of the artery to the lower border of the sinus floor, the diameter of the artery, and the type of artery (intrasinusoidal, intraosseous or superficial) in the first and second premolar and molar regions were measured. The data was analyzed with the t tests, the one-way analysis of variance (ANOVA) and the χ^2 tests.

Results. The maxillary PSAA was recognized in 187 (76.3%) scans. The mean distance between the artery and the floor of the sinus was 6.87 ±3.68 mm. The mean diameter of the artery was 1.37 ±0.61 mm. The greatest mean diameter of the artery was observed in the second premolar region, and the smallest in the first molar region. As many as 63.6% of the arteries were intraosseous, 28.9% intrasinusoidal, and 7.5% superficial.

Conclusions. Due to the high prevalence of the intraosseous type, in most cases of sinus lift surgery there is an increased possibility of PSAA damage. As the largest diameter of the artery was observed in the second premolar region, the possibility of severe bleeding during sinus lift surgery in this area is increased. The average distance between the artery and the floor of the sinus was approx. 7 mm. Consequently, it is recommended that the lower border of the sinus access window should be as high as 7 mm to the floor of the sinus.

Keywords: CBCT, cone-beam computed tomography, maxillary sinus, posterior superior alveolar artery, PSAA

Introduction

Sufficient alveolar bone in terms of width and length is essential for the successful insertion of dental implants. However, the vertical dimension of the bone is reduced in the posterior region of the maxilla due to the pneumatization of the maxillary sinus following tooth loss, making implant placement difficult.[1,2]

The maxillary sinus is pyramidal in shape and retains its overall size when the posterior teeth are in use. However, it expands with age, especially when the posterior teeth are lost. In this case, the sinus cavity expands in both the lower and upper directions. The maxillary sinus cavity is lined with respiratory epithelium, beneath which the connective tissue is immediately adjacent to the bony walls covered with the periosteum. This complex of structures is known as the Schneiderian membrane. Most blood supply to the maxillary sinus and the Schneiderian membrane is provided by the posterior superior alveolar artery (PSAA) and the infraorbital artery (IOA), which are branches of the maxillary artery.[3,4] The maxillary artery itself is a branch of the external carotid artery, which divides into 5 branches in the pterygopalatine fossa.

The PSAA and IOA have intraosseous and extraosseous branches that anastomose about 19 mm from the alveolar bone crest in the anterior-lateral antral bone wall.[3–6] The PSAA travels to the end of the maxillary tuberosity to reach the bone and the periosteum. The location of this artery is usually determined between the lower and middle third of the lateral wall of the sinus.[7] With the development of implant treatment, Tatum and then Boyne introduced a technique called sinus floor elevation, which provides the required bone height to correctly place implants.[1,2]

The most common complication of dental implant insertion in the maxilla is the perforation of the sinus membrane.[8] Bleeding during sinus lift surgery is the second most common complication after the perforation of the Schneiderian membrane, mostly caused by an injury to PSAA.[3] This complication prolongs the surgical process due to reduced vision followed by reduced patient cooperation, and a higher risk of inflammation, pain and infection after surgery. Other reported complications include postoperative bleeding, nose bleeds, nasal congestion, hematoma, empyema, and sinus infection. The adequate pre-surgical and intra-surgical localization of the arteries, particularly of the PSAA, can prevent the injury to these structures and make the surgery less complex.[5,9]

Cone-beam computed tomography (CBCT) is the current diagnostic imaging technique used for the planning of most kinds of dental treatment. In recent years, it has reached extremely high resolution levels. However, this procedure is always to the detriment of the patient, who receives a dose of ionizing radiation. On the other hand, these radiation doses are becoming lower thanks to technological advancements.[10,11] There is increasing evidence that the use of magnetic resonance imaging (MRI) can bring the same effects as CBCT, but with the absence of ionizing radiation, which allows more frequent examinations without biological damage. For now, there is limited availability of this type of imaging for surgical use in dentistry, but in the near future, it may represent an excellent technique for this purpose.[12,13] In the implant treatment plan, CBCT provides valuable information about the width and height of the alveolar bone, bone morphology, pathologies, and important anatomical landmarks.[6,7]

Considering the necessity of familiarity with the anatomy of these arteries and genetic variations in different populations, the present study aimed to evaluate the anatomical position of PSAA by means of using CBCT in a defined group of patients in Qazvin, Iran.

Material and methods

In this descriptive, analytical cross-sectional study, all the maxillary CBCT scans from the archives of the Maxillofacial Radiology Clinic in Qazvin, Iran, were examined by the enumeration method and after reviewing the inclusion criteria, 537 cases were found to be eligible for the study. All patients had signed an informed consent form and gave permission to use their tomographic data anonymously for scientific purposes.

The exclusion criteria were: poor quality of CBCT scans; the presence of sinus pathologies; jaw fractures; the presence of dental implants in the maxillary sinus area; sinus grafts; complete edentulism (bilateral maxilla–mandible in the posterior area), no sinus observations, and duplex incontinence.

The images were taken with the ProMax® 3D imaging unit (Planmeca, Helsinki, Finland) with a voxel size of 0.3 × 0.3 mm^2. In each case, each quadrant was examined separately. In the presence of the teeth, the location of PSAA (in the area of first and second molars as well as first and second premolars) was determined based on the long axis of the teeth.[14] In the edentulous areas, the approximate location of the teeth was determined by the opposite or adjacent teeth, and the long axis of the alveolar crest was drawn.

All measurements were performed separately by 2 trained individuals (an oral radiologist and an implantologist) and the correlation coefficient was 93.6%.

Irradiation conditions, including kVp, mA and time, were adjusted based on the patient size. The observers examined the CBCT images in the sagittal plane by using the Romexis® Viewer, v. 3.8.3 (Planmeca, Helsinki, Finland). In all cases, the vertical distance from the lower border of the artery to the lower border of the sinus floor (Fig. 1), the diameter of the artery (Fig. 2) and the type of artery (intrasinusoidal, intraosseous or superficial) in the first and second premolar and molar areas (Fig. 3) were examined and categorized based on age and gender.

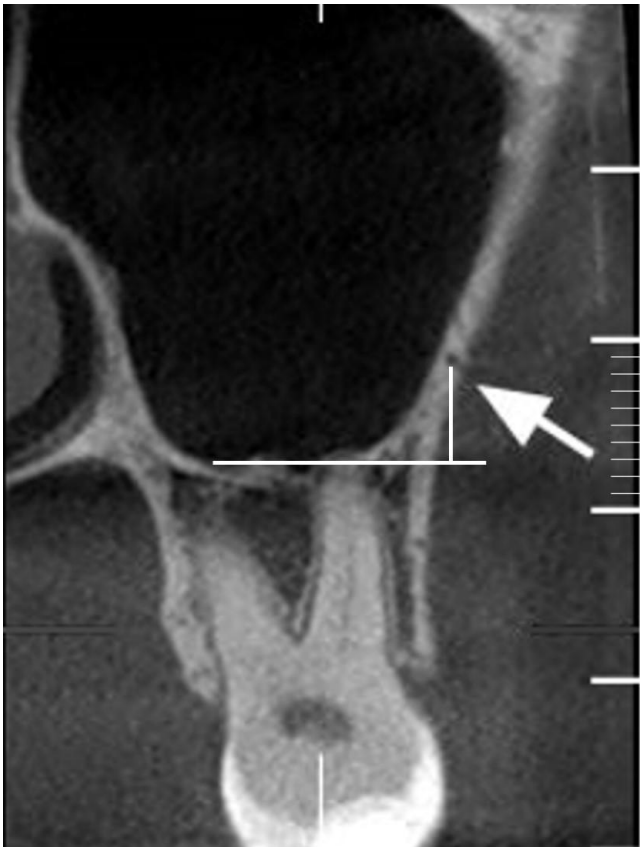

Fig. 1. Measurement of the vertical distance from the lower border of the artery to the lower border of the sinus floor

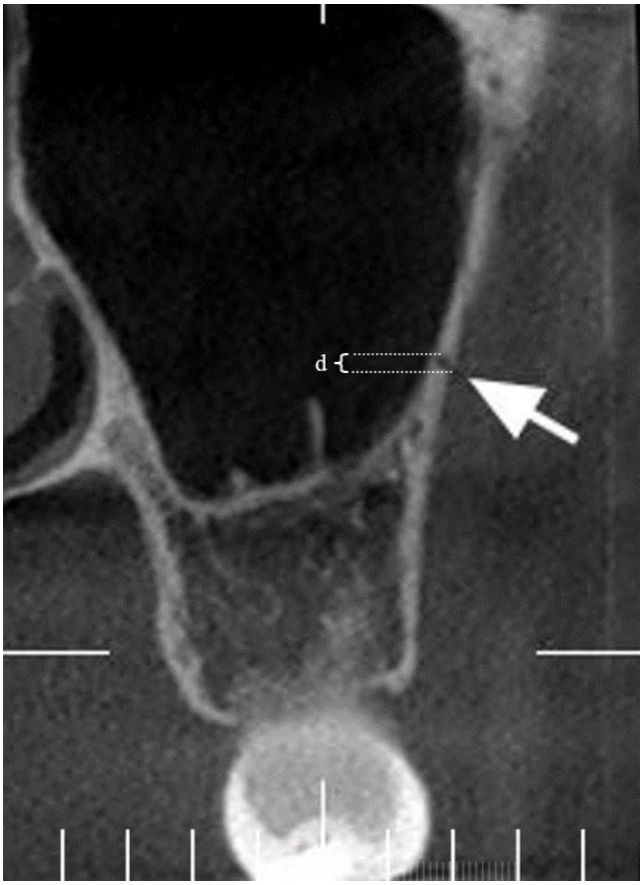

Fig. 2. Measurement of the diameter of the artery

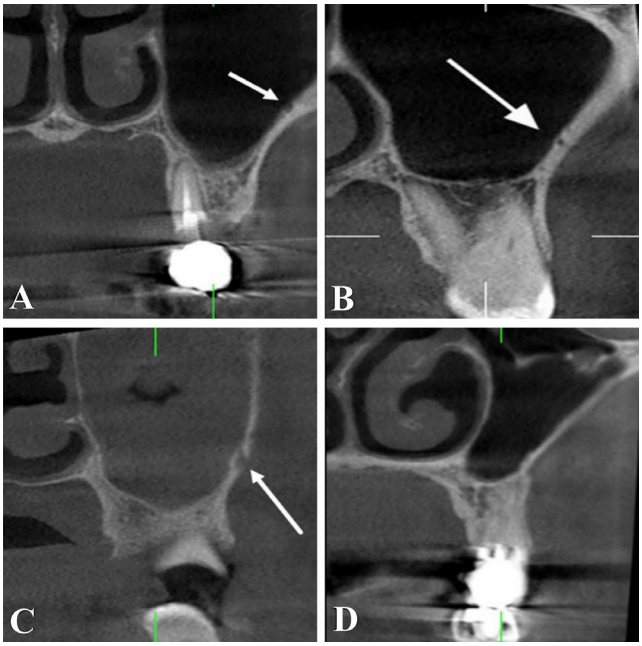

Fig. 3. Type of artery
A – intrasinusoidal; B – intraosseous; C – superficial.

Statistical analysis

The data was analyzed with the IBM SPSS Statistics for Windows software, v. 21.0 (IBM Corp., Armonk, USA). The Kolmogorov–Smirnov test was used to evaluate the normality of data distribution. Descriptive results were calculated by frequency, mean (*M*) and standard deviation (*SD*), based on the type of variable. The independent *t* tests and the one-way analysis of variance (ANOVA) were used to investigate the relationships between the quantitative variables. The χ^2 tests were used to investigate the relationships between qualitative variables. The level of significance was set at $p < 0.05$.

Results

A total of 245 CBCT scans from 80 males (32.7%) and 165 females (67.3%) met the eligibility criteria of this study. The patients were aged 17–68 years, with an average age of 38.46 years. Among the 245 samples, PSAA was recognized in 187 cases (76.3%), but not observed in 58 cases (23.7%).

The χ^2 tests were used to explore the relationships between age and the visibility of PSAA. There was a significant relationship between the arterial observation and age ($p = 0.040$). The highest rate of observation was noted in the age range of 40–50 years, and the lowest rate at the age of <30 years (Table 1).

The PSAA was observed in 77.5% of men and 75.8% of women. There was no significant difference between the rate of observation and gender ($p = 0.400$).

Table 1. Relationship between the rate of observation of the posterior superior alveolar artery (PSAA) and age

Age [years]	Rate of observation *n* (%)		*p*-value
	visible	not visible	
<30	40 (63.5)	23 (38.5)	0.040*
30–39	66 (79.5)	17 (20.5)	
40–50	47 (82.5)	10 (17.5)	
>50	34 (81.0)	8 (19.0)	

* statistically significant.

The mean distance from the artery to the sinus floor was 6.87 ±3.68 mm. The longest distance from the artery to the floor of the sinus was in the second molar region, and the shortest in the second premolar region. The mean distances obtained in the areas of second premolars, first premolars, second molars, and first molars were 6.52 mm, 6.58 mm, 7.43 mm, and 6.85 mm, respectively. There were no significant differences in the distances to the sinus floor in the different areas (p = 0.790).

In male patients, the mean distance from the artery to the floor of the sinus was 6.49 mm, and in females it was 7.2 mm. No significant difference was found between males and females in this respect (p = 0.950). The mean distances from the artery to the sinus floor in age groups <30 years, 30–39 years, 40–50 years, and >50 years were 6.44 mm, 6.01 mm, 7.64 mm, and 6.94 mm, respectively. The relationship between the distance and age was not significant (p = 0.800).

The mean diameter of the artery was 1.37 ±0.61 mm. The largest mean diameter of the artery was observed in the second premolar region, and the smallest in the first molar region. No significant relationships were found between the artery diameter and the dental area (p = 0.800) or age (p = 0.630) (Table 2). In addition, the difference between the mean artery diameter in men (0.35 ±1.33 mm) and women (0.41 ±1.49 mm) was not significant (p = 0.070).

The PSAA was intraosseous in 63.6% of cases, intrasinusoidal in 28.9% of cases, and superficial in 7.5% of cases.

Table 2. Differences in the diameter of the posterior superior alveolar artery (PSAA) between different dental areas and age groups

Variable		PSAA diameter [mm]	*p*-value
Dental area	second premolar	1.44 ±0.39	0.800
	first premolar	1.37 ±0.59	
	second molar	1.37 ±0.88	
	first molar	1.25 ±0.40	
Age [years]	<30	1.40 ±0.20	0.630
	30–39	1.40 ±0.41	
	40–50	1.39 ±0.44	
	>50	1.28 ±0.30	

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

The highest distribution was related to the intraosseous type, and the lowest to the superficial type. No significant differences were found between the artery type and age or gender (p > 0.05) (Table 3).

Table 3. Distribution of the posterior superior alveolar artery (PSAA) type according to age and gender

Variable		PSAA type	Percentage [%]	*p*-value
Age [years]	<30	intraosseous	65.0	>0.05
		intrasinusoidal	25.0	
		superficial	10.0	
	30–39	intraosseous	60.6	
		intrasinusoidal	31.8	
		superficial	7.6	
	40–50	intraosseous	61.7	
		intrasinusoidal	36.2	
		superficial	2.1	
	>50	intraosseous	70.6	
		intrasinusoidal	17.6	
		superficial	11.8	
Gender	male	intraosseous	66.1	>0.05
		intrasinusoidal	27.4	
		superficial	6.50	
	female	intraosseous	62.4	
		intrasinusoidal	29.6	
		superficial	8.0	

Discussion

Bleeding from PSAA is the second most common complication during sinus lift surgery. The occurrence of this complication can restrict vision and access during surgery, leading to the perforation of the sinus membrane or the inadequate removal of the Schneiderian membrane.

The percentage of arterial observation with the use of CBCT in the current study was 76.3%, which agrees with the studies of Chitsazi et al. (71.0%)[15] and Khojastehpour et al. (80.6%).[16] Rosano et al. reported 47% for this rate,[17] Güncü et al. 64.5%,[18] Mardinger et al. 55.0%,[19] Ilgüy et al. 89.3%,[20] and Anamali et al. 93.9%.[21] Regarding these differences in the percentage of observation, several factors can be considered. First, some of these studies used CT to assess the arteries, and it is known that CBCT provides more accurate linear assessment than CT. Also, the observer's skill in examining radiographs could be considered a contributing factor. In the present study, 2 observers were involved for more accuracy. In addition, some of these studies used printed CBCT images for the measurements. Thus, it was not possible to change the magnification and contrast for more accurate assessment. Finally, the most important factor related to these discrepancies may be racial differences.

In the present study, a significant relationship was noted between the rate of the arterial observation and age. The highest rate of observation was seen in the age range of 40– 50 years, and the lowest at the age <30 years. This indicates that aging increases the likelihood of artery recognition on radiographs, which may be attributed to an increased probability of the presence of osteoporosis in the sinus walls with aging.

There was no significant difference between the arterial observation rate and gender, which is consistent with Khojastehpour et al.[16] and Ilgüy et al,[20] but in contrast to Kim et al.[22]

There are 3 types of PSAA: intraosseous; intrasinusoidal; and superficial. In this study, the prevalence of intraosseous, intrasinusoidal and superficial arteries was 63.6%, 28.9% and 7.5%, respectively. It should be noted that in all age and gender groups, the most common type was an intraosseous artery, and the least common the superficial type. These findings agree with the observations of Danesh-Sani et al.,[23] Ilgüy et al.,[20] Güncü et al.,[18] and Chitsazi et al.[15]

The alveolar ridge undergoes changes after tooth extraction, which mainly depend on the anatomy and quality of the bone. From the age of 20, the maxillary sinus reaches its final size, about 5 mm below the nasal cavity. The protruding dental roots are associated with a thin cortex in the apex region and after the extraction of the root, a great deal of resorption occurs in the buccal apical region. However, the reduction of the ridge dimensions is particularly due to the trauma caused by tooth extraction rather than the pneumatization of the maxillary sinus.[15]

In most studies, the distance between the artery to the apex of the alveolar ridge has been evaluated, but the sinus floor is a more suitable starting point for this measurement, as it changes less than the alveolar ridge. Therefore, in the present study, the distance from the artery to the sinus floor in the areas of first and second molars and premolars was evaluated.

In the current study, the mean distance from the artery to the sinus floor was 6.87 ±3.68 mm. The longest distance was observed in the second molar region, and the shortest in the second premolar region. These findings are also in line with the results of Danesh-Sani et al.[23] and Jung et al.[24] The distance in Danesh-Sani et al.'s study was about 8.17 mm,[23] and in Jung et al.'s study it was 8.8 mm.[24] These values are a little higher than those obtained in the present study and the discrepancy may be attributable to racial differences and the number of samples. It indicates that the distance of PSAA from the second molar region to the first molar and second premolar regions decreases, but it increases in the first premolar region due to the more cranial position of the sinus floor and a higher artery position relative to the infraorbital cavity.

In previous studies, the distance from the artery to the floor of the sinus was evaluated based on age and gender. In the present study, it was found that there were no significant differences in the distance from the artery to the floor of the sinus between the age and gender groups.

In this study, the mean diameter of PSAA was 1.37 ±0.61 mm, with the largest diameter observed in the second premolar region, and the smallest in the first molar region. In a study by Danesh-Sani et al., the average diameter was 1.17 mm,[23] in Chitsazi et al.'s study it was 1.37 mm,[15] and in Güncü et al.'s study it was 1.3 mm,[18] all of which are in agreement with the present results. All of these previous studies did their evaluations using CBCT images. Studies using CT reported larger diameters, indicating differences in the measurement instruments. Researchers that evaluated the diameter of PSAA in cadaver, such as Ella et al.[25] and Hur et al.,[26] reported diameters of 1.2 mm and 0.8 mm, respectively, which are smaller than the diameters reported in CBCT studies. There is a direct relationship between the artery diameter and the likelihood of bleeding during sinus lift surgery. Consequently, with diameters greater than 2 mm, the probability of bleeding increases.[27] According to the results of the present study, as well as those of previous studies, a diameter greater than 2 mm is not common.

No significant difference was observed between the artery diameter and gender, which agrees with the results obtained by Mardinger et al.[19] and Chitsazi et al.,[15] but is in contrast to Khojastehpour et al.[16]

In this study, no significant difference was found between the artery diameter and age, which agrees with Danesh-Sani et al.,[23] but is in contrast to Khojastehpour et al.[16] and Güncü et al.,[18] where the relationship between the diameter and age was significant. In these latter studies, it was found that as age increases, the diameter of the artery increases as well. The reason for these differences across studies may be attributed to the age range in Khojastehpour et al.'s study, which was 25–86 years, with an average of 46 years, which was higher than in this study. We recommend that further studies use a larger sample size, and similar age and gender groups.

Limitations

The limitation of this study was the lack of a uniform distribution for the studied age groups. Thus, differences in the visibility of PSAA within each group were not evaluated separately.

Conclusions

In all age and gender groups, the most common type of PSAA was the intraosseous type. Therefore, due to the high prevalence of the intraosseous type, in most cases of sinus lift surgery there is an increased possibility of PSAA artery damage. The largest artery diameter was observed in the second premolar region. Consequently, the possibility of severe bleeding during sinus lift surgery is the highest in this area. Based on these results, we suggest to provide the lower border of the sinus access window as high as 7 mm to the floor of the sinus.

Ethics approval and consent to participate

The research was approved by the institutional Ethics Committee at Qazvin University of Medical Sciences, Iran. Written informed consent was obtained from all participants.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Questionable accuracy of CBCT in determining bone density: A comparative CBCT–CT in vitro study

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Abstract

Background. The accuracy of the estimation of radiological bone density with the use of the cone-beam computed tomography (CBCT) grayscale is still questionable. Standardization and correlation with the gold standard computed tomography (CT) Hounsfield units (HU) is required prior to clinical application.

Objectives. The objective of the present study was to evaluate the reliability of the grayscale for the estimation of bone density, using samples with intact soft tissue in order to substantiate the clinical use of the scale.

Material and methods. A total of 240 sites in 20 goat heads were scanned to obtain radiological bone mineral density via Hounsfield units in CT and the grayscale in CBCT. The anatomical architecture of soft tissues was preserved for all samples. Two observers obtained the data, which consisted of 3 variables (mean, minimum and maximum) for both scales. The statistical analysis of the data was conducted using Cronbach's alpha, Pearson's correlation coefficients, the independent samples *t* tests, and regression analysis.

Results. Differences in the means of the mean, minimum and maximum values between the 2 scales were statistically highly significant ($p = 0.000$). The correlation coefficients for the mean, minimum and maximum values of the 2 scales were 0.496, 0.037 and 0.396, respectively. Regression analysis revealed that the R^2 values for the mean, minimum and maximum values were 29.79%, 21.05% and 19.45%, respectively.

Conclusions. The positive but weak correlation between the 2 scales and the low predictive reliability of the grayscale reveals its questionable applicability for the estimation of density in comparison with the standard HU.

Keywords: CT, CBCT, bone density, Hounsfield units, grayscale

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Introduction

Constant remodeling due to physiological processes and aging phenomena have immensely increased the need to study bone quality and its importance while planning surgical and non-surgical procedures. The rehabilitative management of post-menopausal osteoarthritis and osteoporosis, fracture healing, orthodontic correction, and implant-supported occlusion rehabilitative surgeries include the radiological estimation of bone mineral density as an integral part of procedure planning and execution.[1]

The superimposition and consequent camouflage of the bony landmarks by anatomical structures compromise the visualization of regions of interest (ROI) in two-dimensional (2D) radiography. Such limitations are overcome by precise quantitative and qualitative imaging techniques, such as computed tomography (CT). Bone density is represented by Hounsfield units (HU) on CT scans, and is considered the standard for qualitative bone estimation.[2] The HU value depends on several factors, including the composition and nature of the tissue being imaged.[3]

Medical CT is commonly used for the imaging of several pathologies. This technique provides high precision and better visualization of hard tissues, making it a favorable approach for the evaluation of mineral density. High radiation exposure and the high cost of CT scans underscore the need for advances in this imaging modality. Comparable high resolution with low radiation exposure and cost-efficiency can be achieved by using cone-beam computed tomography (CBCT).

The calibration of the grayscale is done by taking HU as the standard base scale for the evaluation of mineral density. The HU value used in CT is calculated from the linear attenuation coefficient of each tissue and takes the linear attenuation coefficient of water into account. The grayscale also shows a linear relationship with the attenuation coefficient of the scanned material.[4] The reliability of the grayscale for the evaluation of mineral density has been a subject of discussion, as there have been many studies performed to assess the correlation between HU and the grayscale, with varying results.[5–7]

The available literature demonstrates the use of cortical and trabecular bone equivalent materials, along with the soft tissue simulations done by homogenous resin coverage, in experiments to derive mineral density. The homogeneity of the bio-like material, along with the absence of multi-level anatomical architecture in soft tissue imitations, fails to replicate heterogeneous human tissue, which can be a roadblock for the application of such bio-like materials in clinical scenarios.[8] It has been established that the attenuation of radiation is a direct linear function of the density of the matter. Furthermore, it is certain that the data formulated while working with dried preserved tissues cannot be compared with human tissues of variable densities due to their complex architecture. The varied densities of fat, vessels, hair and skin, and hard and soft connective tissues, are important variables, considering the attenuation of the radiation beam.[9] Even though a positive correlation has been revealed in previous studies, several authors have mentioned the need for the inclusion of living physiological tissue rather than using cadaveric mandibles and tissue equivalent materials for the sake of the attenuation offered by the adherent soft tissue in the actual patient.[8]

The present study aimed to evaluate the accuracy and applicability of the grayscale values of CBCT as compared to the HU of CT in estimating radiological bone mineral density. The study further aimed to obtain the conversion ratios for transmuting the available CBCT grayscale into the gold standard HU in order to establish a standardized scale for density estimation.

Material and methods

This study was conducted in the Department of Oral Medicine and Radiology at the I.T.S. Centre for Dental Studies and Research, Ghaziabad, India, and at the Advanced Diagnostic Center, Hargobind Enclave, Delhi, India, to evaluate the accuracy of the CBCT grayscale in determining bone density. The study was approved by the institutional Ethics Committee (ITSCDSR/IIEC/2018-21/OMR/OMR). The radiological mineral density of the specific anatomical areas in the goat mandible and maxilla at the same spatial coordinates was measured using CBCT (grayscale) and CT (HU).

Goat heads with intact physiological soft tissue were acquired from the local butcher. The goats were butchered for the purpose of consumption. To preserve the integrity of soft tissues, the goat heads were stored at a temperature of 4°C in an icebox. The scans were done on the same day when the samples were acquired.

The mean, minimum and maximum values were obtained for each ROI in the CBCT and CT scans. Two radiologists procured the data for both types of scans. A radiologist with an experience of more than 10 years obtained the initial values for both scales, which were then confirmed by another radiologist with 3 years of experience.

During scanning, the occlusal plane of the goat head was kept parallel to the floor. A customized wooden stool was made to standardize the position of the goat heads during CBCT. Sterilization was maintained by using a mackintosh sheet and a transparent cellophane sheet while the head was placed in the CBCT scanner.

A total of 20 samples were included in the study. The axial sections of each sample obtained with CBCT and CT were used for the evaluation. A total of 240 sites were evaluated in 20 samples (i.e., 12 sites for each sample; 6 in the maxillary arch and 6 in the mandibular arch).

The ROI in the maxilla were as follows: the region anterior to first premolar; the region posterior to first premolar; and the region distal to second molar. The ROI in

the mandible were as follows: the region anterior to first premolar; the region posterior to first premolar; and the region distal to the last present molar.

The sites were evaluated bilaterally to obtain the grayscale values from the CBCT scan and HU from the CT scan.

First premolar was used as a standardized marker to ensure the overlapping of the focused sites from both the scans of an individual sample. The sections were adjusted to obtain the largest vertical dimension of first premolar in the sagittal and coronal sections bilaterally in the respective arch. The ROI was then analyzed for the grayscale values and HU from the CBCT and CT scans, respectively. To determine the bone density values of the ROI, a region of an area of 12 mm^2 was traced on to all sites adjacent to the respective tooth. In case of the absence of the reference tooth, the posterior tooth was used as a reference.

The CBCT scans were taken using the NewTom® GiANO scanner (model SN 70820432; Cefla, Imola, Italy,) with the following specifications: field of view (FoV) of 11 × 8 cm^2; slice thickness of 0.15 mm; tube voltage of 90 kVp; tube current of 32 mA; and exposure time of 9 s (pulsed). The CT scans were obtained using the Somaton® scanner (Siemens, Erlangen, Germany) with the following specifications: 120 kV; 118 mA; high-resolution kernel; slice thickness of 0.75 mm; interval of 0.5 mm; pitch of 0.5 mm; and gray density 31.78 bit.

Statistical analysis

The statistical analysis was carried out using the IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA). The inter-observer reliability was calculated using Cronbach's alpha and intraclass correlation coefficients (*ICC*). The means for the mean, minimum and maximum values of the grayscale from CBCT and HU from CT were compared using the independent samples *t* tests and Pearson's correlation coefficients. Scatter plots were prepared using the Minitab® statistical software, v. 19 (Minitab, State College, USA). Regression analysis was performed to examine the relationship between the grayscale (independent variable) and HU (dependable variable) in relation to the mean, minimum and maximum values.

Results

The present in vitro study comprised a total of 20 samples. For each sample, 12 sites (6 maxillary and 6 mandibular) were measured for the grayscale values from the CBCT scans and for HU from the CT scans with the aim of the qualitative estimation of radiological bone mineral density. For every site, the mean, minimum and maximum values for both scales were recorded (Fig. 1–3). Two researchers independently recorded the grayscale values and HU for each sample.

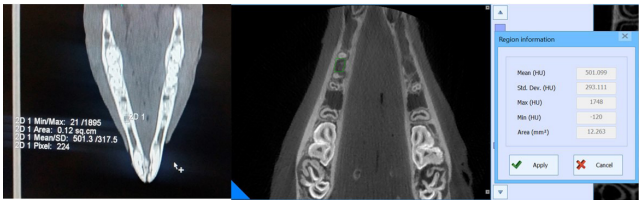

Fig. 1. Placement of the regions of interest (ROI) in the mandibular arch of sample I in the axial section of the computed tomography (CT) scan (left) and in the corresponding axial section of the cone-beam computed tomography (CBCT) scan (right)

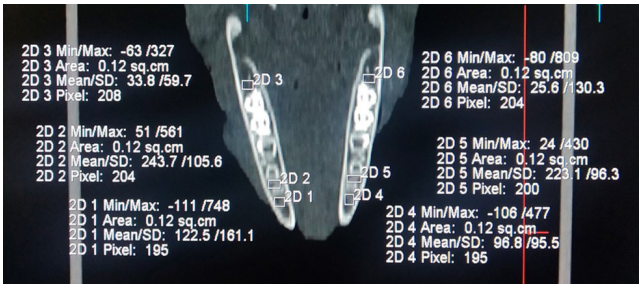

Fig. 2. Axial section of the CT scan of sample II, indicating multiple placements of regions of interest (ROI)

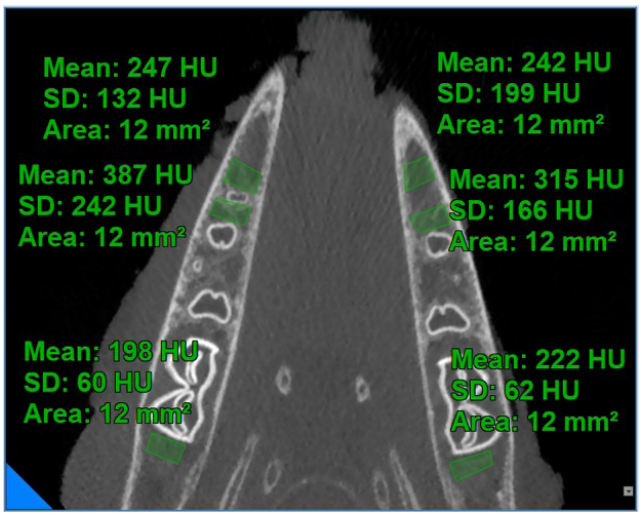

Fig. 3. Axial section of the CBCT scan of sample II indicating multiple placements of regions of interest (ROI)

The statistical analysis of the recorded values revealed an overall *ICC* of 0.979 (97.9%). The evaluation of the inter-observer data reliability with Cronbach's alpha revealed excellent agreement (99%).

The means for the mean, minimum and maximum values for the CT HU were 257.25 ±186.71, 15.18 ±122.61 and 720.98 ±338.07, respectively. The mean values for the mean, minimum and maximum values for the CBCT grayscale values were 355.14 ±188.13, –47.63 ±214.18 and 1,142.39 ±457.72, respectively (Table 1). The statistical analysis using the independent samples *t* test revealed statistically highly significant differences for all 3 sets ($p = 0.000$).

Table 1. Comparison of means between computed tomography (CT) and cone-beam computed tomography (CBCT) for different values

Value	CT *M* ±*SD*	CBCT *M* ±*SD*	*MD* ±*SD*	*t*-value	*p*-value
Minimum	15.18 ±122.61	−47.63 ±214.18	62.81 ±91.57	3.943	0.000*
Mean	257.25 ±186.71	355.14 ±188.13	97.89 ±1.42	5.722	0.000*
Maximum	720.98 ±338.07	1,142.39 ±457.72	421.41 ±119.65	11.473	0.000*

M – mean; *SD* – standard deviation; *MD* – mean difference; * statistically significant (independent samples *t* test).

Pearson's correlation analysis was applied to all 3 value sets (i.e., mean, minimum and maximum values) to establish the relationships between the 2 scales. The analysis revealed that the correlation coefficients for the mean, minimum and maximum values of the 2 scales were 0.496, 0.037 and 0.396, respectively. The analysis further revealed that the correlation between the maximum and mean values of the 2 scales was statistically significant (p = 0.000), but non-significant for the minimum values (p = 0.566). The correlations between the mean CBCT grayscale values and the mean, minimum and maximum CT HU were 0.496, 0.426 and 0.410, respectively, demonstrating a statistically significant correlation between the 2 scales in relation to the respective mean values (p = 0.000) (Table 2).

Table 2. Correlation between CT and CBCT

Correlation		CBCT min	CBCT mean	CBCT max
CT min	*r*	0.037	0.426	0.349
	p-value	0.566	0.000*	0.000*
CT mean	*r*	0.016	0.496	0.464
	p-value	0.804	0.000*	0.000*
CT max	*r*	0.117	0.410	0.396
	p-value	0.070	0.000*	0.000*

min – minimum; max – maximum; *r* – Pearson's correlation coefficient; * statistically significant (two-tailed *t* test).

Furthermore, the scatter plot revealed a strong linear correlation between the HU minimum and the grayscale maximum and mean values, whereas the correlation with the grayscale minimum values was a non-linear relationship (Fig. 4). Similarly, the scatter plot for the HU mean values revealed a strong linear correlation with the grayscale maximum and mean values, and a weak correlation with respect to the minimum values (Fig. 5). The scatter plot for the HU maximum values revealed a strong linear correlation with the grayscale maximum and mean values, but a weak correlation with the minimum values (Fig. 6).

The weak correlation coefficients obtained here reveal the poor reliability of the grayscale for predictions, i.e., for the known CBCT grayscale values, the prediction of the standard CT HU for radiological mineral density is not very reliable.

The findings further reveal that, even though the grayscale accuracy is questionable in relation to the density estimation for all 3 registered variables, there is a comparatively higher reliability of the CBCT grayscale mean values for the prediction of all 3 (i.e., minimum maximum and mean) variables of CT HU.

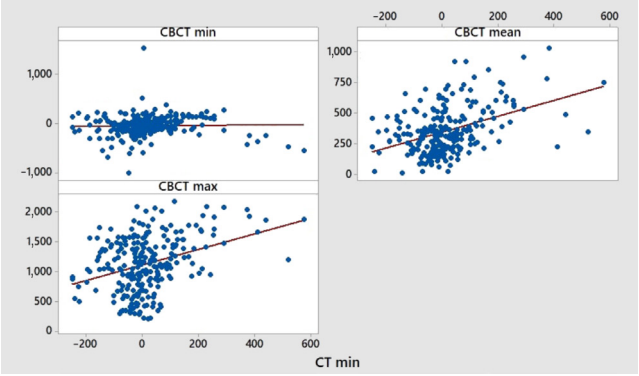

Fig. 4. Scatter plot of the CBCT minimum, CBCT maximum and CBCT mean values vs. the CT minimum values

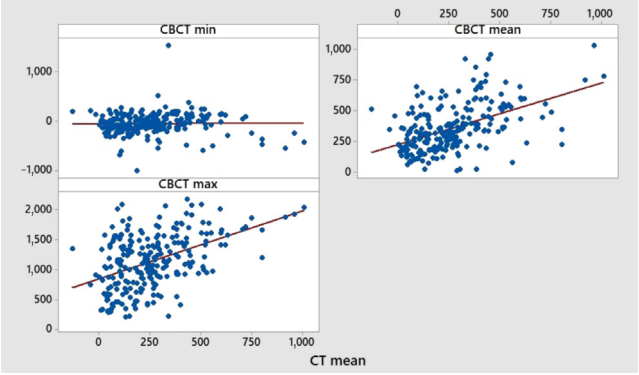

Fig. 5. Scatter plot of the CBCT minimum, CBCT maximum and CBCT mean values vs. the CT mean values

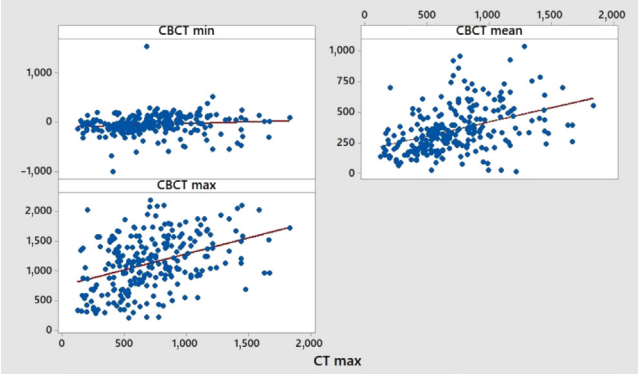

Fig. 6. Scatter plot of the CBCT minimum, CBCT maximum and CBCT mean values vs. the CT maximum values

Regression analysis was done to determine the coefficient of determination for all 3 variables (Table 3). The regression equations for the prediction of CT HU (dependable variable) from the available CBCT grayscale values were as follows (Equations 1–3):

$$\begin{aligned}\text{CT min} = {} & -110.9 - 0.1028\ \text{CBCT min} \\ & + 0.3176\ \text{CBCT mean} \\ & + 0.0073\ \text{CBCT max}\ (R^2 = 21.05\%)\end{aligned} \quad (1)$$

$$\begin{aligned}\text{CT mean} = {} & 9.8 - 0.1816\ \text{CBCT min} \\ & + 0.4933\ \text{CBCT mean} \\ & + 0.0557\ \text{CBCT max}\ (R^2 = 29.79\%)\end{aligned} \quad (2)$$

$$\begin{aligned}\text{CT max} = {} & 366.9 - 0.014\ \text{CBCT min} \\ & + 0.484\ \text{CBCT mean} \\ & + 0.1590\ \text{CBCT max}\ (R^2 = 19.45\%)\end{aligned} \quad (3)$$

As the mean values of the CBCT grayscale showed the highest prediction capability with the lowest chances of error for the prediction of the minimum, maximum and mean values of the CT HU, the regression equations in regard to the CBCT grayscale mean value were as follows (Equations 4–6):

$$\text{CT min} = -83.5 + 0.2777\ \text{CBCT mean}\ (R^2 = 18.16\%) \quad (4)$$

$$\text{CT mean} = 82.3 + 0.4927\ \text{CBCT mean}\ (R^2 = 24.65\%) \quad (5)$$

$$\text{CT max} = 459.5 + 0.736\ \text{CBCT mean}\ (R^2 = 16.79\%) \quad (6)$$

The low R^2 shows a low reliability of the predicted CT HU obtained from the available CBCT grayscale values for the minimum, maximum and mean.

Discussion

Non-physiological tissues or cadaveric samples without the preservation of the adherent soft tissues may produce grayscale values that deviate from the actual values. This is one of the significant concerns for the clinical applicability of CBCT for the estimation of radiological bone mineral density. The available literature has demonstrated a strong positive correlation between the standard protocols and several prediction models for estimating mineral density by means of CBCT. The samples used in such studies range from dried mandibles and phantoms to homogenous density inserts.[5,6] The present study used preserved physiological tissue and its anatomical architecture, addressing the substantial variation offered by the various components of biological tissues, which artificial simulations cannot duplicate. Parsa et al. emphasized that anatomical soft tissue simulations affect the attenuation values while measuring the mineral density.[5] Furthermore, the same authors expressed concern regarding the presence of high-density metal markers and restorative materials, leading to qualitative shortcomings, such as streaking, high noise ratios and beam-hardening artifacts, causing histogram shift and unreliable grayscale values. Previous studies have also stated that, if a varied range of background materials is used, it is quintessential to include the attenuation offered by soft tissues when correlating the 2 scales for clinical application,[7,10–12] which is the issue addressed in the current study.

Previous studies have used a varied array of restorative materials and metal markers to overlap the sections obtained via different imaging techniques. In the present study, an anatomical site was used as a landmark for overlapping ROI in both scans from the same sample, which is unique in comparison with the available literature. A CBCT scanner has a highly sensitive receptor, with the ability to differentiate subtle changes in attenuation,[9] and evidence indicates that the grayscale is subject to variabil-

Table 3. Regression analysis

Dependent variables	Regression equations	R^2 [%]
CT min	CT min = 16.19 + 0.0213 CBCT min	0.14
	CT min = –83.5 + 0.2777 CBCT mean	18.16
	CT min = –91.6 + 0.0935 CBCT max	12.18
	CT min = –110.9 – 0.1028 CBCT min + 0.3176 CBCT mean + 0.0073 CBCT max	21.05
CT mean	CT mean = 257.9 + 0.0141 CBCT min	0.03
	CT mean = 82.3 + 0.4927 CBCT mean	24.65
	CT mean = 41.1 + 0.1892 CBCT max	21.51
	CT mean = 9.8 – 0.1816 CBCT min + 0.4933 CBCT mean + 0.0557 CBCT max	29.79
CT max	CT max = 729.8 + 0.185 CBCT min	1.37
	CT max = 459.5 + 0.736 CBCT mean	16.79
	CT max = 387.1 + 0.2923 CBCT max	15.66
	CT max = 366.9 – 0.014 CBCT min + 0.484 CBCT mean + 0.1590 CBCT max	19.45

ity according to the density of objects in ROI.[4] There is great concern regarding the use of high-density objects and restorative materials as reference points, as they may cause a shift in the determined grayscale values of the sample.[5] The presence of implants and metal markers cause abrupt changes in densities. The variety of densities around the scanned object results in beam hardening, along with other possible associated phenomena, such as the scattered radiation and projection data-related effects, which make the applicability of the grayscale values questionable.[2,13] The use of an anatomical marker in our study assures that the retrieved values are more reliable.

The reliability of the CBCT grayscale has been depicted by either a coefficient of determination (R^2) or a correlation coefficient (r) in previous studies. The available literature mentions the presence of huge bias values and no agreement with regard to applicability.[5] In contrast, the present study considered values form both scales to derive relevant evidence for the suitability of the CBCT grayscale for estimating bone mineral density. The r coefficient for the mean, minimum and maximum values was 0.496, 0.037 and 0.396, respectively, and the R^2 coefficient for the mean, minimum and maximum variables were 29.79%, 21.05% and 19.45%, respectively.

In their review, Pauwels et al. mentioned the questionable potential of considering the correlation coefficient alone, for there can be a significant deviation from the linear fit (coefficient of determination) for statistically significant correlations.[4] It has also been mentioned that for substantiating the clinical use of the grayscale values, both r and R^2 have to be taken into account, and the values are not interchangeable. Both the values of Pearson's correlation coefficient (r) and the coefficient of determination (R^2) considered in our study further validate the disputed use of the grayscale for density estimation.

In contrast to the current results, Casetta et al. reported that differences between the CBCT voxel values (VV) and the CT (HU) gray density values were statistically significant ($p < 0.05$).[14] In addition, an r-value of 0.978 demonstrated a statistically significant linear correlation between VV and HU gray density values.[14]

The current study shows a positive but weak correlation between the 2 scales, not per the previous literature. The statistical analysis showed that among the 3 variables that were compared, the highest correlation (r = 0.496, R^2 = 29.79%) was observed for the relationship between the mean values of the 2 scales, which implies that the mean values of the grayscale are the most reliable for the evaluation of density.

According to de Carvalho Crusoé Silva et al., the mean HU value obtained using CBCT (418.06) was significantly higher than that obtained using multi-slice computed tomography (MSCT) (313.13).[15] Thus, the authors concluded that bone density in HU obtained from the CBCT images proved unreliable, since it was higher than that obtained using MSCT.[15] Similarly, the differences between the minimum, mean and maximum values noted in our study were 62.81 ±91.57, 97.89 ±1.42 and 421.41 ±119.65, respectively. Differences between all the variable values were statistically significant, and thus question the applicability of the grayscale for density estimation.

Challenges regarding the applicability of the grayscale are also likely due to shortcomings in the basic radiation physics principle, beam geometry, and the assumptions and limitations of the available reconstruction algorithms. The artifacts resulting from variability in the axial plane (e.g., cupping and doming artifacts), beam hardening, the concepts of endomass and exomass, the divergence of the beam, and a high noise ratio in CBCT scans are also a matter of concern in terms of measuring radiological bone mineral density.[4]

Similar to the present study, comparative studies performed for the standardization of the grayscale used a limited number of scanning protocols and a limited variety of machine models. In addition, the array of scanning protocols and the models used fluctuate in the literature. Varshowsaz et al. conducted an in vitro study to compare the density values of different tissue phantoms, with 2 different thicknesses, 2 different image acquisition settings and 3 locations in the phantoms.[16] The analysis found significant differences between the density values obtained from the CBCT and CT scans in most situations. Furthermore, the CBCT values were not similar to the CT values in any of the phantoms using different thicknesses and acquisition parameters, or the 3 different sites, which is analogous to our findings. The abovementioned authors also stated that machine-related factors could be responsible for the questionable reliability reported in the literature, supporting the applicability of the grayscale for radiological bone density estimation.[16]

The use of a single scanning protocol and a single machine model can be considered as shortcomings of the present study, and may provide a scope for future detailed studies. The obtained data, even when showing a strong positive correlation, cannot be applied to multiple available models due to the characteristic variation in device calibration and the scanning protocol used for imaging.[16,17] Even for the same machine, the imaging protocol has a varied range according to the required radiological insights. Image acquisition requires different settings and changes in calibration, which can be manually adjusted or automatically applied, depending upon the model in use.[2]

The accuracy of density estimation with a smaller FoV is greater than with a larger FoV due to the high resolution of the target area. The largest FoV available was used while scanning the goat heads, which is a shortcoming of the present study. On the other hand, a small FoV can result in reduced density values, as the diameter of the X-ray beam decreases. This may lead to a decrease in the number of low-energy photons and an increase in the penetration of radiation, which leads to a reduction in the attenuation of X-rays, and finally the gray values.[2]

Another way in which FoV affects the evaluation of density involves the concept of exomass. The exomass is the mass that is present outside the dimensions of FoV during image acquisition. The literature shows that variability in the amount of exomass evaluated in different FoV sizes is related to the gray values.[4] Preserved physiological tissue architecture provides a non-homogenous exomass in close proximity to ROI, which can produce variation in the grayscale values and HU provided by natural tissue. Candemil et al., while studying the effects of non-homogenous hyperdense artifacts in homogenous regions, concluded that not only was there a great discrepancy in the data obtained by 3 machines, but also in the distribution of hyperdense artifacts from the exomass that followed the inherent gray value dispersion of CBCT images, with less homogeneity in the inner zone of FoV.[18] This is exacerbated when metal objects are in the exomass. Katsumata et al. found that a greater FoV eliminates the exomass, resulting in less variability in the gray values,[19] which can justify the use of a large FoV in the present study.

Conclusions

The mean and maximum variables of the grayscale were positively but weakly correlated with HU, whereas the minimum values showed a non-significant correlation. While the correlation is positive, the predictive reliability of the grayscale is low for HU. With the current results as evidence and a review of the available literature, it can be concluded that the accuracy of the CBCT grayscale in measuring bone density, in contrast to CT HU, is questionable and needs to be standardized before clinical application.

Ethics approval and consent to participate

The study was approved by the institutional Ethics Committee at the I.T.S. Centre for Dental Studies and Research, Ghaziabad, India (ITSCDSR/IIEC/2018-21/OMR/OMR).

Data availability

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

Consent for publication

Not applicable.

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Original paper

Evaluation of the effect of the photobiomodulation therapy on the pain related to dental injections: A preliminary clinical trial

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Abstract

Background. Pain from dental injections is a common reason why people fear dentistry and avoid dental treatment. Thus, researchers have attempted to find methods to decrease dental injection pain.

Objectives. Considering the analgesic effect of the photobiomodulation therapy (PBMT), the aim of this study was to evaluate the effects of PBMT on the pain caused by dental anesthetic injections.

Material and methods. This randomized, split-mouth, triple-blind clinical trial evaluated 60 bilateral canine teeth in 30 dental students. After the random selection of the test (laser) quadrant, the injection site was irradiated with a 940 nm diode laser. Buccal infiltration anesthesia was then administered by injecting lidocaine plus epinephrine with a short needle. The level of pain experienced during the injection was determined using a 100-millimeter visual analog scale (VAS). The same procedure was performed for the control (no laser) quadrant, with the difference being that the laser handpiece was turned on, but no radiation was administered. The 2 groups were compared using the non-parametric Wilcoxon signed-rank test.

Results. The mean VAS pain scores were 21.2 ±15.7 for the laser quadrant and 27.9 ±18.9 for the control quadrant; this difference was statistically significant ($p = 0.030$), but did not seem to be clinically relevant.

Conclusions. The photobiomodulation therapy prior to dental anesthetic injections has no clinical advantage for reducing injection pain.

Keywords: pain, local anesthesia, laser, injection, low-level laser therapy

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Introduction

Pain is defined as an unpleasant feeling that is experienced when actual tissue damage or trauma with the potential to cause tissue damage occur.[1] Pain from dental anesthetic injections has always been a challenge during dental treatment. Evidence has shown that the fear of dental injections is a major reason why people avoid dental visits and dental care services, which can adversely affect a person's oral health status.[2] Dental injection pain can be due to the mechanical trauma caused by the insertion of the needle into the tissue, the release of the anesthetic agent into the tissue or the removal of the needle. Several factors can affect the level of anesthetic injection pain, such as the type of anesthetic agent injected, the gauge of the needle, and the temperature and pH of the injected agent.[3] Topical anesthetic agents in the form of anesthetic gels and sprays have been proposed to reduce injection pain. Some simple techniques, such as the compression and precooling of the injection site, have also been suggested for this purpose.[4,5] Moreover, some fundamental advances have been made in anesthetic injection techniques in recent years, such as the use of computerized injection systems, which aim to reduce anesthetic injection pain and improve the patient's comfort. However, further studies are required to develop painless anesthetic injection techniques that can be applied for widespread use in dental offices.[6]

In recent years, the photobiomodulation therapy (PBMT) and the application of biostimulative lasers have gained prominence in this field. In this treatment modality, the laser beam is able to penetrate deeply into the tissue due to its low output power, which is usually <500 mW, and the selected laser wavelengths, which are usually between 630 nm and 1,300 nm. In PBMT, the absorbed energy does not heat or damage the living tissue; instead, the energy of the laser photons is absorbed by the cells and stimulates them, which is referred to as photostimulation.[7] The photobiomodulation therapy has several advantages at the cellular level, including the metabolic activation of the cells and the improvement of their function, the stimulation of the repair process, anti-inflammatory effects, analgesic effects due to the release of endorphins, the stimulation of the immune system, and an increased anti-oxidative capacity of the blood.[8]

Over the years, since its approval by the U.S. Food and Drug Administration (FDA) as a pain reduction modality, PBMT has become increasingly popular.[9,10] Although the exact mechanism of action of PBMT for pain reduction is not completely understood, evidence supports the idea that PBMT activates certain peripheral analgesic mechanisms that can affect pain perception. Thus, the PBMT protocols for pain control have been studied under different conditions.[11] The analgesic effects of PBMT in the management of pain following endodontic surgery, temporomandibular joint pain, trigeminal neuralgia, myalgia, aphthous ulcers, and tooth hypersensitivity have previously been confirmed.[12] However, the effect of PBMT on pain from anesthetic injections in the oral cavity has not been thoroughly examined. Thus, the aim of this study was to evaluate the effect of PBMT on pain from dental anesthetic injections.

Material and methods

This randomized, split-mouth, triple-blind clinical trial included 30 candidates who were selected from dental students at Tehran University of Medical Sciences, Iran. The study was conducted in the Laser Department of the university.

The study was approved by the institutional Ethics Committee at Tehran University of Medical Sciences (IR.TUMS.DENTISTRY. REC. 1397.067) and registered in the Iranian Registry of Clinical Trials (IRCT2019042904418N1). All patients signed informed consent forms prior to their participation in the study, and they were free to withdraw at any point during the study.

A total of 60 sound maxillary canine teeth were selected bilaterally. The teeth had no carious lesions, periapical lesions, extensive restorations, or anatomical anomalies or cracks; teeth with any of these problems were excluded from the study. The participants were healthy and had no cardiovascular diseases, thyroid disorders, or any other systemic conditions. There could be no history of taking antibiotics or nonsteroidal anti-inflammatory drugs (NSAIDs) in the past 2 months, which would contraindicate lidocaine plus epinephrine injections.

Considering the results of a pilot study on 3 samples and using the feature for comparing two means with the Minitab® Sample Size Calculation software (Minitab, State College, USA), the minimum sample size was calculated to be 22. The following were assumed: $\alpha = 0.05$; $\beta = 0.2$, standard deviation of the mean of 5, and a minimum difference between the 2 mean values of 4.4. Thirty patients were recruited to ensure accuracy and an adequate sample size. Since the study had a split-mouth design, the statistician randomly selected the test (laser) quadrant by flipping a coin and reported the result to the laser specialist.

In the test (laser) quadrant, the handpiece of a semiconductor diode laser (Epic 10; BIOLASE Inc., Foothill Ranch, USA) was positioned at the buccal vestibule of the maxillary canine and the injection site, with a cross-sectional area of 0.785 cm^2. It was irradiated with 15.28 J/cm^2 energy density, 200 mW power, and 940 nm wavelength for 60 s, using the continuous-wave mode. The anesthetic was injected immediately after the removal of the laser tip by means of the buccal infiltration anesthesia technique with a short 27-gauge needle (Soha, Tehran, Iran). Half of the anesthetic cartridge containing 2% lidocaine plus epinephrine 1:100,000 (Persocaine-E; Darou Pakhsh, Teh-

ran, Iran) was injected. The bevel of the needle faced the bone at the site of the apex of the canine tooth at an angle of 45° and the anesthetic agent was injected at a speed of 1 mL/min. All injections were performed by the same operator to ensure optimal quality and consistency, and to eliminate the confounding effect of interindividual differences in experience and expertise.

Immediately after the completion of the injection in the test quadrant, the level of pain experienced by the participant during the injection was quantified using a 100-millimeter visual analog scale (VAS). The participants had been instructed on how to use the scale earlier.

The same procedure was performed for the control quadrant, with the difference being that the laser handpiece was turned on, but no radiation was administered.

The participants, the operator who performed the injections and the examiner who recorded the VAS pain scores were all blinded to the laser and control quadrants (triple-blind design).

Statistical analysis

For statistical analysis, the IBM SPSS Statistics for Windows software, v. 21.0 (IBM Corp., Armonk, USA), was used.

The measures of central dispersion, such as mean (*M*), standard deviation (*SD*), and minimum and maximum levels of pain according to VAS, were calculated for the laser and control groups. Since the pain score was an ordinal variable and the pain score data was not normally distributed, the 2 groups were compared using the nonparametric Wilcoxon signed-rank test. A p-value <0.05 was considered statistically significant.

Results

A total of 30 patients between 22 and 30 years of age (with a mean age of 26.5 years) were evaluated in this study. Twenty (66.7%) were males, and 10 (33.3%) were females. The level of pain was measured using VAS. A VAS score of 0 indicated no pain at all, while a score of 100 indicated the most excruciating pain imaginable. Table 1 presents the measures of central dispersion of the pain scores.

The distribution was assessed using the Shapiro–Wilk normality test, and it was determined to be not normal. According to the Wilcoxon signed-rank test, the mean VAS pain score in the laser group was significantly lower than in the control group (p = 0.030).

Table 1. Measures of central dispersion of the dental injection pain score determined with a 100-millimeter visual analog scale (VAS)

Variable	Group	*M* ±*SD*	Minimum	Maximum
Pain	laser	21.1 ±15.7	0.0	60.0
	control	27.9 ±18.9	0.0	70.0

M – mean; *SD* – standard deviation.

Discussion

Anesthesia through injection is an essential aspect of most kinds of dental treatment. Patients seeking dental treatment are often concerned about anesthetic injections and the pain they cause. Painless anesthetic injections can increase the patient's comfort and cooperation, positively affect the quality of treatment, and build the patient's trust. Thus, several methods have been suggested to reduce dental anesthetic injection pain.[13,14] Considering the favorable biological effects of PBMT, including its analgesic action,[8] this study evaluated the influence of PBMT on the pain caused by dental anesthetic injections.

The results of this study indicate that applying PBMT prior to an anesthetic injection significantly decreased the level of pain experienced during the injection. In vivo studies on the effects of PBMT on oral mucosal innervation have shown that PBMT decreases the frequency of pain signals transferred by the nerve fibers and increases the stimulation threshold of the nerve fibers.[7] Laser has an inhibitory effect on A-delta and C fibers, and consequently decreases the speed of transfer of pain signals, lowers the action potential and inhibits neurogenic inflammation.[15] In this study, the pain reduction experienced on the laser side can be attributed to changes in the synthesis, release and metabolism of the chemical mediators of pain in the peripheral nervous system.[16]

Evidence indicates that PBMT can be used as an effective non-pharmaceutical modality for pain relief following nonsurgical endodontic treatment.[17,18] In their study on 10 children between 6 and 9 years of age, Tanboga et al. evaluated the effect of a low-level erbium-doped yttrium aluminum garnet (Er:YAG) laser on the pain caused by cavity preparation.[19] They concluded that patients who underwent PBMT prior to cavity preparation experienced a lower level of pain during this process.[19] Shapiro et al. reported that the application of a low-level Er:YAG laser in a laser anesthesia device along with lidocaine caused a significant reduction in needle insertion pain in intramuscular injections.[20] This finding was attributed to the destruction of the stratum corneum layer through PBMT, which consequently enhanced the penetration and faster action of lidocaine.[20] In addition, it has been demonstrated that the use of aluminum gallium arsenide (AlGaAs) PBMT along with regional intravenous anesthesia can decrease the level of pain experienced during and after treatment.[11] Jagtap et al. determined that PBMT prior to anesthetic injections for tooth extraction caused a significant reduction in the level of pain experienced during the injection.[3]

On the contrary, some studies have reported that PBMT did not have any clinical advantage with regard to pain reduction in dental procedures.[21,22] Payer et al. evaluated the effects of PBMT on inflammation, the course of healing and pain following endodontic surgical procedures, and concluded that there were no significant differences

between the laser and placebo groups in any of the assessed parameters.[23] There are a number of factors, such as radiation dosage, wavelength, exposure time, the type of tissue, and the optical properties of the laser, that can affect the efficacy of PBMT in pain control.[3,8] In addition, variability in the results of studies can be due to different treatment protocols.

In 2016, Ghaderi et al. evaluated the effect of PBMT on the pain caused by dental anesthetic injections and demonstrated that patients who received topical anesthetic gel along with PBMT prior to anesthetic injections reported significantly lower VAS pain scores as compared to those who received topical anesthetic gel alone; however, this difference was not clinically significant.[14] Similar results were obtained in the present study. Although the mean pain score was significantly lower in the laser group, this difference may not have been clinically relevant, as several studies have determined that a minimum change of 13–30 mm in the VAS score is needed in order to achieve clinical significance.[24] In this study, the difference in the mean pain scores between the 2 groups was below this range.

This prospective clinical trial had a split-mouth design, which eliminated the confounding effects of interindividual differences. Moreover, considering the blinding of the participants to the laser side and the random allocation of the laser and control sides, the carry-over effect, which is an inherent drawback of split-mouth studies,[25] was eliminated in this design.

This study used a triple-blind design. The dental clinician who performed the anesthetic injections and the examiner who recorded the pain scores were not aware of which side underwent irradiation. In addition, the laser handpiece light was turned on for both the laser and control sides. Furthermore, laser irradiation did not cause any visual or auditory stimulation, did not create heat, and had no vibration.[26] Thus, the patients were not aware of which side received PBMT.

To quantify the level of pain experienced, VAS was employed in the present study. This scale was used due to its simplicity and compatibility with different populations and study designs. Its application does not require a training course and it can be easily completed in less than 1 min. Moreover, evidence has demonstrated that VAS is sensitive to changes in pain perception.[27]

Accelerated collateral circulation as well as an increased blood vessel diameter can be observed with PBMT. Therefore, when PBMT is applied prior to injection, the risk of hematoma formation should be considered.

Laser therapy is a novel field of science, and its application methods and treatment protocols are under constant development. Considering the inconsistency and controversy in the results of other PBMT studies, and the fact that this study had a small sample size and was performed on dental students, further investigations with larger sample sizes from the general public are imperative. Furthermore, due to the small sample size, it was impossible to make a comparison between men and women concerning the VAS scores, or a comparison between the 1st and 2nd injection. Future studies should evaluate the efficacy of PBMT for other dental conditions that cause pain, such as the root canal treatment of inflamed teeth, as well as its potential for use as an alternative to supplement anesthetic injections. It should be noted that the risks and possible complications of laser therapy should be carefully investigated.

Conclusions

The results of the current study indicate that although PBMT with the set parameters had a statistically significant impact on the pain caused by injections with the buccal infiltration anesthesia technique, there was not a considerable clinical effect.

Ethics approval and consent to participate

The study was approved by the institutional Ethics Committee at Tehran University of Medical Sciences, Iran (IR.TUMS.DENTISTRY. REC. 1397.067), and registered in the Iranian Registry of Clinical Trials (IRCT2019042904418N1) Written informed consent was obtained from all participants.

Data availability

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

Consent for publication

Not applicable.

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Original paper

Evaluation of lingual mucosa toxicity and recovery follow-up in rats, following sub-chronic exposure to titanium dioxide nanoparticles

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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. Nanosized titanium dioxide (TiO_2) particles are among the most widely used nanoparticles (NPs) worldwide due to their unique properties. The lingual mucosa is still neglected in terms of risk assessment with respect to the NP uptake.

Objectives. The aim of this study was to evaluate the effect of intragastric administration of TiO_2 NPs on the mucous membranes of the tongues of albino rats, as well as the potential benefits of a 4-week recovery period.

Material and methods. Twenty-one male albino rats were randomly divided into 3 groups ($n = 7$ per group). Group I served as a control group and received saline intragastrically daily for 30 days. The experimental groups included group II, which received TiO_2 NPs in the amount of 50 mg/kg of body weight (b.w.) intragastrically daily for 30 days, and group III, which also received TiO_2 NPs in the amount of 50 mg/kg b.w. intragastrically daily for 30 days, and then was allowed to recover for 4 weeks. Tongue specimens were collected for histological, immunohistochemical and morphometric examinations.

Results. The TiO_2 NP group II showed significant atrophic and degenerative changes in the tongue mucosa, which included reduced epithelial thickness in the ventral surface, with disfigurement in the filiform and fungiform papillae. Weak B cell lymphoma-2 (Bcl-2) immunoreactivity was also noted. The 4-week recovery group displayed improvement in the histological picture, with moderate to strong Bcl-2 immunoreactivity in the epithelial cell layers and the underlying connective tissue.

Conclusions. The use of TiO_2 NPs caused severe histological and apoptotic changes in the filiform and fungiform papillae, and the ventral surface of the tongue of the rats, while allowing recovery minimized the toxic effect of the NPs.

Keywords: titanium, titanium dioxide, nanoparticle, Bcl-2, rats

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Introduction

Continuous improvement in nanotechnology engineering has led to the extensive manufacturing and use of nanomaterials. Usually, nanomaterials are nanosized solid particles with a diameter ranging from 1 nm to 100 nm. Among the popular metals and metal oxides occurring in the nanosized form are gold, silver, iron, and titanium oxide nanoparticles (NPs), which are widely used due to their superior physicochemical properties, such as optical features and magnetic activity, and high thermal and electrical conductivity. Another favorable characteristic is their large surface area to volume ratio.[1,2] However, their escalating consumption raises concerns with regard to their impact on biological systems.[3]

Titanium dioxide nanoparticles (TiO_2 NPs) are widely used, as they exhibit unique properties, including antiviral, antibacterial and antifungal effects. Moreover, TiO_2 NPs are highly biocompatible, with great strength, corrosion resistance and low density.[4,5] However, their specific large surface area and quantum effects increase their chemical reactivity in the human body. Strikingly, the incorporation of TiO_2 NPs in numerous products, such as food additives, cosmetics and dental care products, raises the exposure risk related to these NPs. Owing to their non-degradability and ability to accumulate in different tissues, elevated exposure to TiO_2 NPs may lead to chronic cytotoxicity.[6,7]

Previously published research reports that TiO_2 NPs exert toxic effects on the human body. These include changes in the cell cycle, damage to the nuclear membrane and apoptosis.[8,9] Additionally, it has been proposed that TiO_2 NPs might cause mitochondrial and DNA destruction within cells.[10,11] The mechanism of TiO_2 NP toxicity can be summarized in 3 processes: (1) the production of reactive oxygen species (ROS)[12]; (2) damage to the cell wall and the lipid peroxidation of the cell membrane; and (3) the attachment of TiO_2 NPs to intracellular organelles and biological macromolecules, following damage to the cell membrane.[13]

Exposure to TiO_2 NPs can occur by many routes, including inhalation, injection, dermal and mucosal contact, and intragastric absorption. This can lead to the precipitation of TiO_2 NPs in different internal organs.[14,15] Weir et al. reported that the highest amount of TiO_2 as particles sized <100 nm was found in candies and chewing gum.[16] Thus, such products expose the oral mucosa to high doses of TiO_2.

The aim of the present study was to investigate the histopathological changes in the filiform and fungiform papillae, and the ventral surface of the tongue, following the intragastric administration of TiO_2 NPs in albino rats, as well as the possible effect of recovery after the withdrawal of the NPs.

Material and methods

Chemicals

Titanium dioxide NPs were purchased from Nano Gate, Cairo, Egypt. The TiO_2 powder consisted of 95–97% anatase phase and 3–5% brookite phase, with NPs of an average size of 15 ±3 nm and a spherical shape (based on transmission electron microscopy (TEM)). The TiO_2 anatase particles were prepared by precipitation from a homogeneous solution, using titanium (IV) isopropoxide as a precursor in an aqueous solution acidified with nitric acid to a pH of 2, with a water-to-titanium mole ratio of about 200.[17]

Animals

Twenty-one male albino rats weighing 150–200 g were used in the study. The rats were 2 months old, which is equivalent to the age of a human child. Children are susceptible to consuming up to 4 times more TiO_2/kg of body weight (b.w.) as compared to adults.[18]

Experimental protocol

The experiment was conducted according to the guidance and approval of the Ethical Committee at the Faculty of Dentistry of Ain Shams University, Cairo, Egypt (approval No.: 615/2017).

The animals were provided by and housed in the Animal Research Center of Ain Shams University. The animals were housed in a sterile, controlled environment, with a temperature of 29 ±2°C and 12-hour dark/light cycles. They were kept in individual cages, with 5 rats per cage. The size of the cage was 20 cm in width and 40 cm in length. The cages were cleaned on a daily basis, and the rats were allowed free access to food and water. Each rat was given a unique number. The rats were randomly and equally allocated into 3 main groups (n = 7):

– group I: the rats received saline intragastrically daily for 30 days to serve as controls;
– group II: the rats received TiO_2 NPs in the amount of 50 mg/kg b.w. intragastrically daily for 30 days[19]; and
– group III: the rats received TiO_2 NPs in the amount of 50 mg/kg b.w. daily for 30 days as in group II, and were then allowed to recover for 4 weeks after treatment.

The rats were euthanized with an intracardiac overdose of sodium thiopental (80 mg/kg b.w.), following the different experimental periods.[20]

Postmortem specimen processing

The rats' tongues were excised and fixed immediately in 10% phosphate-buffered formaldehyde solution for 48 h. The specimens were washed adequately under run-

ning water, dehydrated by transferring through a series of graded alcohol, cleared in xylene, and embedded in paraffin wax according to the standard technique.[21]

Hematoxylin and eosin staining

Longitudinal sections, 5-micron-thick, were obtained. The right sides of the tongues were stained with hematoxylin and eosin (H&E).[21] A light microscope (Leica, Wetzlar, Germany) with ×400 magnification was used to obtain images that were transferred to a computer system for analysis. The epithelial thicknesses of the filiform and fungiform papillae, and the ventral surface of the tongue were measured (in microns) using the ImageJ software, v. 1.41a (National Institutes of Health (NIH), Bethesda, USA; https://imagej.nih.gov/ij/).

Immunohistochemical staining

The left sides of the tongue tissue specimens (formalin-fixed and paraffin-embedded) were used for anti-active B cell lymphoma-2 (Bcl-2) localization.[22] The polyclonal primary rabbit anti-active Bcl-2 supplied by Biocare Medical (Pacheco, USA) (1:100 in phosphate-buffered saline (PBS)), code No. CP 229 A, was applied. All sections were counterstained with hematoxylin, incubated for 2–3 min, rinsed in distilled water, dehydrated in graded alcohol, and cleared in xylene. Finally, the specimens were mounted by using an aqueous mounting medium, cover slips were put in place and the samples were examined with a light microscope.

For immunohistochemical evaluation, the Bcl-2-stained cell surface area percentage was measured using the ImageJ software, v. 1.41a.

Statistical analysis

The mean (*M*) and standard deviation (*SD*) values for the epithelial thickness (μm) and the Bcl-2 mean area percentage were analyzed using the IBM SPSS Statistics for Windows software, v. 26.0 (IBM Corp., Armonk, USA). The one-way analysis of variance (ANOVA) and Student's *t* test were used to compare the data. A *p*-value equal to or less than 0.05 was considered statistically significant.

Results

Histopathological results (H&E staining)

Filiform papillae

Group I (control)

The control group specimens showed a normal dorsum surface of the tongue with abundant slender filiform papillae that were regularly arranged and covered with keratinized stratified squamous epithelium. These papillae presented a uniform arrangement of the epithelial cell layers, and the keratin layer was evident. There were dispersed clear cells. The underlying lamina propria revealed normal cellular and fibrous elements, and appeared to merge with the subjacent tongue muscles without a clear line of demarcation (Fig. 1A).

Group II (TiO_2 NPs)

As compared to group I, there was noticeable disfigurement in the structure of the filiform papillae, with a marked reduction in their height; they exhibited blunt-ended tops.

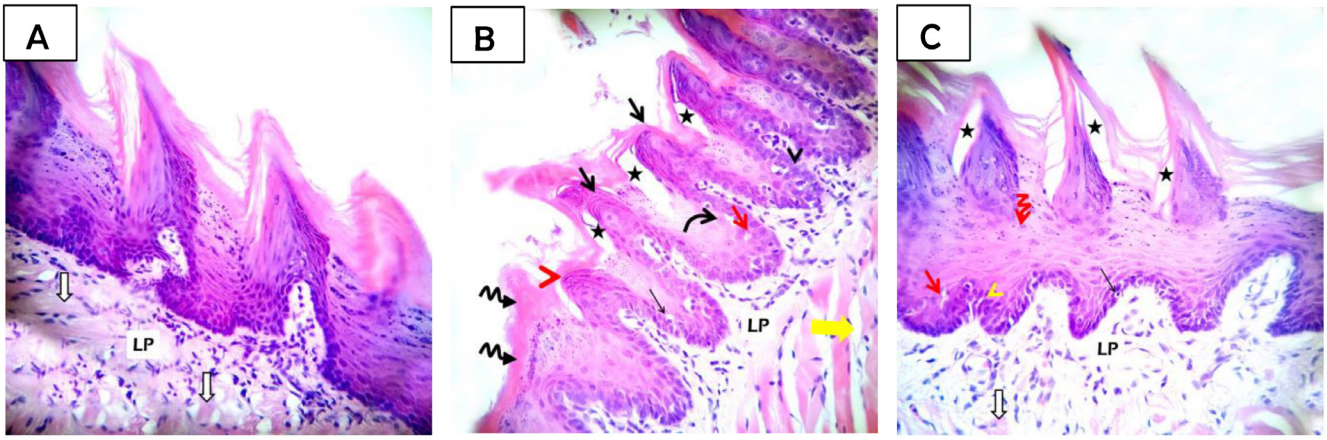

Fig. 1. Photomicrographs of the dorsal surface of the rat tongue, demonstrating the filiform papillae (hematoxylin and eosin (H&E), ×400 magnification)

A – group I showing normal, cone-shaped keratinized filiform papillae with a normal architecture of the lamina propria (LP) and tongue muscles (white arrows); B – group II showing disfigured papillae with blunt-ended tops (black arrows), a short papilla (red arrow head), the loss of the papillae with hyperkeratosis (wavy black arrows), the loss of the papillae with evident hyperkeratosis (curved black arrow), focal areas of separation in the keratin layer (black stars), the epithelium with basilar hyperplasia (black arrow head), cytoplasmic vacuolation (red arrow), clear cells (thin black arrow), inflammatory cell infiltrate in the lamina propria (LP), and separation between muscle fibers (yellow arrow); C – group III showing the papillae seemingly regaining their normal appearance; still, with the epithelium revealing mild basilar hyperplasia (yellow arrow head), binucleated cells (curved red arrow), cytoplasmic vacuolation (red arrow), clear cells (thin black arrow), focal areas of separation in the keratin layer (black stars), and inflammatory cell infiltrate in the lamina propria (LP).

Also, some areas revealed a complete loss of the papillae with evident hyperkeratosis. Focal separation of the keratin layer from the underlying epithelial cells was also observed. The basal cell layer appeared disorganized, with basilar hyperplasia and some nuclear alterations in the form of pyknosis. Some of the basal and suprabasal epithelial cells had vacuolated cytoplasm. Numerous clear cells were recognized. The lamina propria showed signs of degeneration, with moderate inflammatory cell infiltration. Separation between tongue muscle fibers was noted (Fig. 1B).

Group III (TiO_2 NPs with 4-week recovery)

The tongues of the rats that were allowed a recovery period of 4 weeks after 30 days of TiO_2 NP induction were similar to those in the control group in the histological picture of the surface epithelium and the lamina propria. The epithelium appeared to have a considerable thickness and a regular basement membrane. The basal cell layer exhibited mild basilar hyperplasia and large hyperchromatic nuclei. The prickle cell layer displayed a few binucleated cells. The granular cells exhibited distinct keratohyalin granules, but a few cells appeared with vacuolation. The keratin layer was irregular, had nonuniform thickness in certain areas, and had focal areas of separation. A few clear cells were identified throughout the epithelial layers. The underlying lamina propria showed less degenerative areas, deeply stained basophilic fibroblasts and a small population of inflammatory cells (Fig. 1C).

Fungiform papillae

Group I (control)

The fungiform papillae were of normal mushroom shape and were composed of normal stratified squamous epithelium with a thin uniform layer of keratin and a vascular connective tissue core. A single barrel-shaped taste bud with peripherally arranged cells was observed on the upper surface of the papillae. Lingual muscle fibers ran in different directions (Fig. 2A).

Group II (TiO_2 NPs)

The fungiform papilla was shorter and distorted, and showed signs of epithelial atrophy. Atrophic changes in the shape and orientation of the taste bud cells, which were separated, were also observed. Atrophy in the connective tissue and tongue musculature was noted (Fig. 2B).

Group III (TiO_2 NPs with 4-week recovery)

Similar to the control group, the fungiform papillae assumed a mushroom-like appearance, with almost normal epithelial covering and a barrel-shaped taste bud with normal cells. Reduced signs of degeneration within the lamina propria and the blood vessels engorged with blood cells were observed. Lingual muscle fibers appeared to run in different directions (Fig. 2C).

Ventral surface of the tongue

Group I (control)

The mucous membrane of the ventral surface of the tongue had a regular, thin keratin layer that lacked the lingual papillae. The lamina propria was formed of the connective tissue. Muscle fibers were composed of the interlacing bundles running in different directions (Fig. 3A).

Group II (TiO_2 NPs)

The ventral surface of the tongue showed the atrophy of the surface epithelium, observed as a reduction in the thickness of the surface epithelium. An apparent loss of the keratin layer was observed. The dissociation of the collagen fibers of the lamina propria and congested blood vessels were noted. Skeletal muscle fibers were apparently atrophied and separated (Fig. 3B).

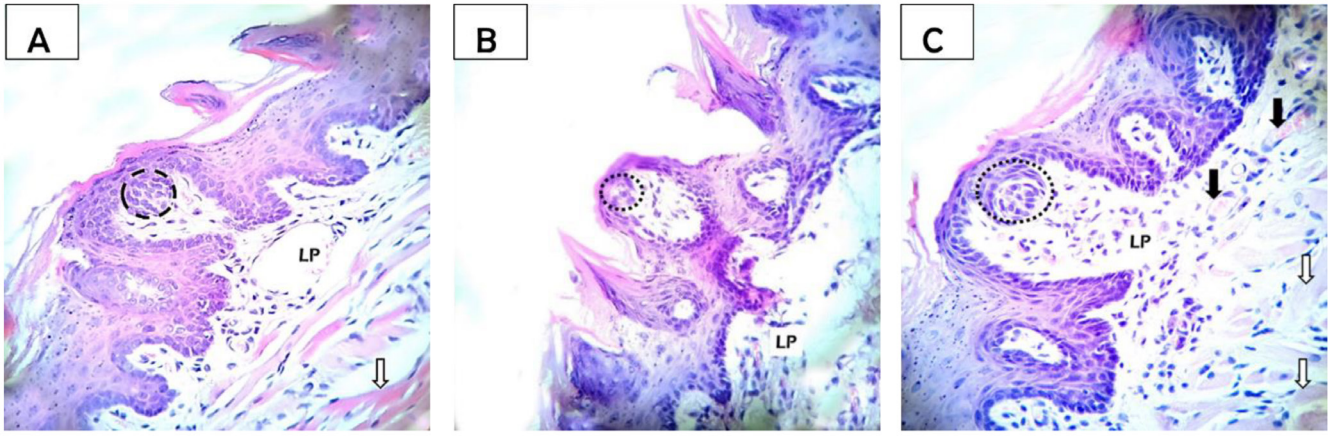

Fig. 2. Photomicrographs of the dorsal surface of the rat tongue, demonstrating the fungiform papillae (hematoxylin and eosin (H&E), ×400 magnification)

A – group I showing a normal mushroom shape of the papilla with a normal barrel-shaped taste bud (dotted circle); B – group II showing the distortion of the papilla with signs of atrophy in a taste bud (dotted circle) and the lamina propria (LP); C – group III showing a nearly normal shape of the papillae and a taste bud with almost a regular arrangement of taste cells (dotted circle), the lamina propria (LP), blood vessels (black arrows), and muscle fibers (white arrows).

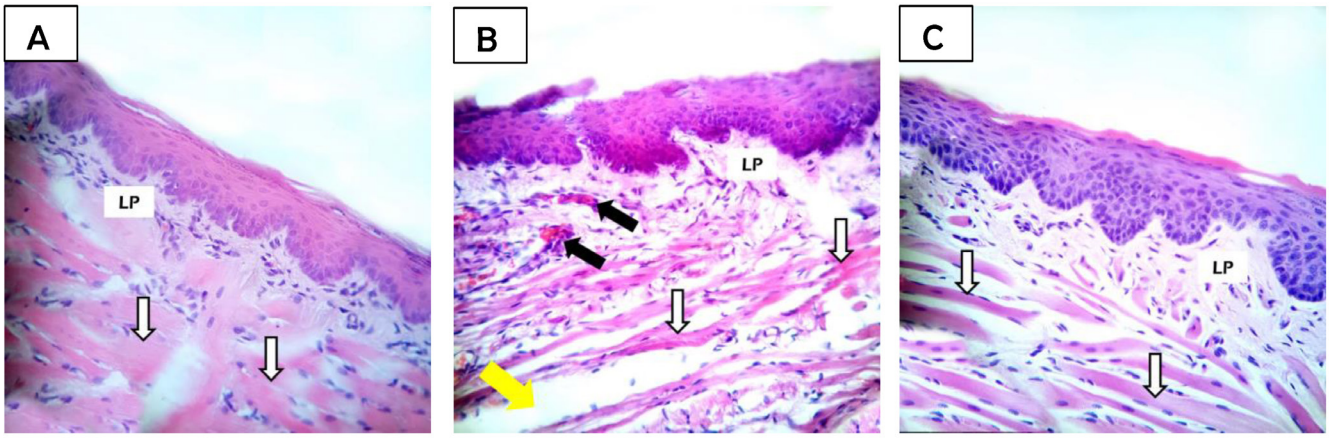

Fig. 3. Photomicrographs of the ventral surface of the rat tongue (hematoxylin and eosin (H&E), ×400 magnification)

A – group I showing no lingual papillae and the presence of a regular, thin keratin layer; B – group II showing an irregular contour with thin epithelium, the loss of the keratin layer, congested blood vessels (black arrows), and atrophied muscle fibers (white arrows) with separation between muscle fibers (yellow arrow); C – group III showing the presence of normal epithelium with a thin keratin layer, muscle fibers (white arrows) and the lamina propria (LP).

Group III (TiO_2 NPs with 4-week recovery)

The ventral surface of the tongue regained its normal surface epithelium thickness, with a thin keratin layer and a normal pattern of epithelial ridges. Lingual muscle fibers ran in different directions (Fig. 3C).

Morphometric analysis

Statistical analysis for the mean epithelial thickness of the filiform and fungiform papillae, and the ventral surface of the tongue in the 3 groups is presented in Tables 1 and Tables 2.

The ANOVA revealed statistically significant differences between the 3 groups in the mean epithelial thickness of the filiform and fungiform papillae, and the ventral surface of the tongue ($p < 0.01$). The highest mean epithelial thickness of the filiform and fungiform papillae, and the ventral surface of the tongue was recorded in group I (control), whereas the lowest mean epithelial thickness was recorded in group II (TiO_2 NPs).

The pairwise comparison conducted with the use of unpaired Student's *t* test revealed a statistically significant decrease in the mean epithelial thickness of the filiform and fungiform papillae, and the ventral surface of the tongue in group II (TiO_2 NPs) as compared to both group I (control) and group III (TiO_2 NPs with 4-week recovery) ($p < 0.01$).

Table 1. Epithelial thickness [µm] of the filiform and fungiform papillae, and the ventral surface of the tongue in the 3 examined groups

Group	Filiform papillae	Fungiform papillae	Ventral surface of the tongue
I	364.03 ±8.81	202.59 ±7.28	118.20 ±13.20
II	163.19 ±12.41	128.77 ±5.79	61.63 ±5.80
III	356.83 ±5.09	196.34 ±2.40	108.50 ±6.70

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

Table 2. Pairwise comparison between the examined groups with regard to the mean epithelial thickness [µm] of the filiform and fungiform papillae, and the ventral surface of the tongue

Variable	Comparison	*MD*	*p*-value
Filiform papillae	group I vs. group II	200.84	<0.010*
	group I vs. group III	7.20	0.090
	group II vs. group III	193.64	<0.010*
Fungiform papillae	group I vs. group II	73.82	<0.010*
	group I vs. group III	6.25	0.052
	group II vs. group III	67.57	<0.010*
Ventral surface of the tongue	group I vs. group II	56.57	<0.010*
	group I vs. group III	9.70	0.100
	group II vs. group III	46.87	<0.010*

MD – mean difference; * statistically significant.

Immunohistochemical results

The immunostained sections obtained from the filiform and fungiform papillae, and the ventral surface of the tongue of group I showed numerous cells with positive Bcl-2 immunoreactivity, indicating a strong reaction affecting the epithelial layers and extending to the underlying connective tissue cells in the form of brownish coloration (Fig. 4A, 5A and 6A). The Bcl-2-immunostained sections of group II demonstrated a marked reduction in positively stained cells, indicating a weak reaction (Fig. 4B, 5B and 6B). The Bcl-2-immunostained sections of group III revealed a moderate-to-strong reaction in the epithelial cell layers and the underlying connective tissue (Fig. 4C, 5C and 6C).

Morphometric analysis

Statistical analysis for the mean percentage of the area of the cells positively stained with Bcl-2 immune stain in all groups for the different examined specimens is shown in Tables 3 and Tables 4.

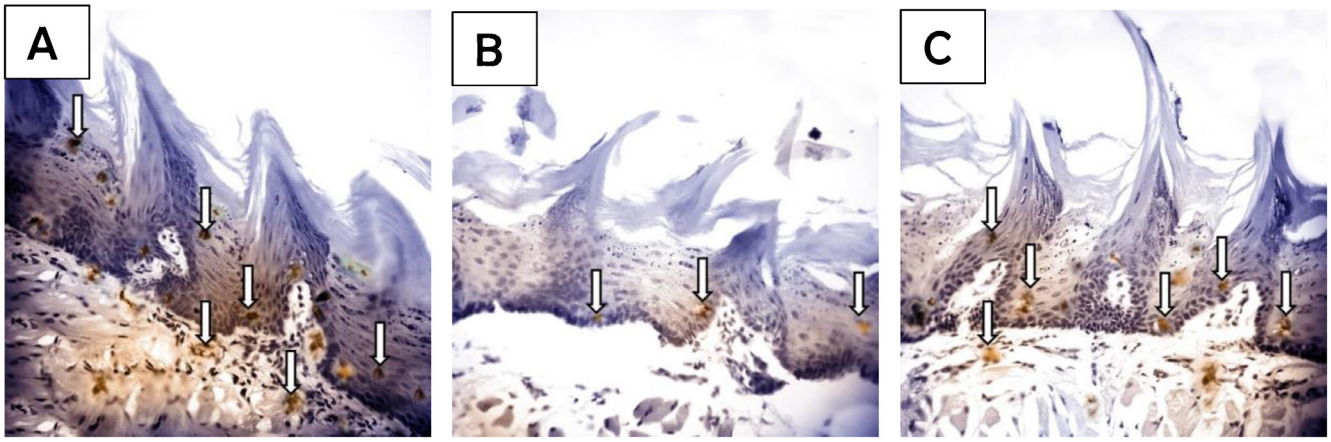

Fig. 4. Photomicrographs of the dorsal surface of the rat tongue, demonstrating the filiform papillae (B cell lymphoma-2 (Bcl-2), ×400 magnification)

A – group I showing a strong cytoplasmic reaction to Bcl-2 in several epithelial layers and the underlying connective tissue; B – group II showing a negative cytoplasmic reaction in most epithelial cells except for few positive cells; C – group III showing a moderate-to-strong cytoplasmic reaction to Bcl-2 in the basal cell layer, which is evident in some prickle cells, with few positive cells in the connective tissue. White arrows indicate positively stained cells.

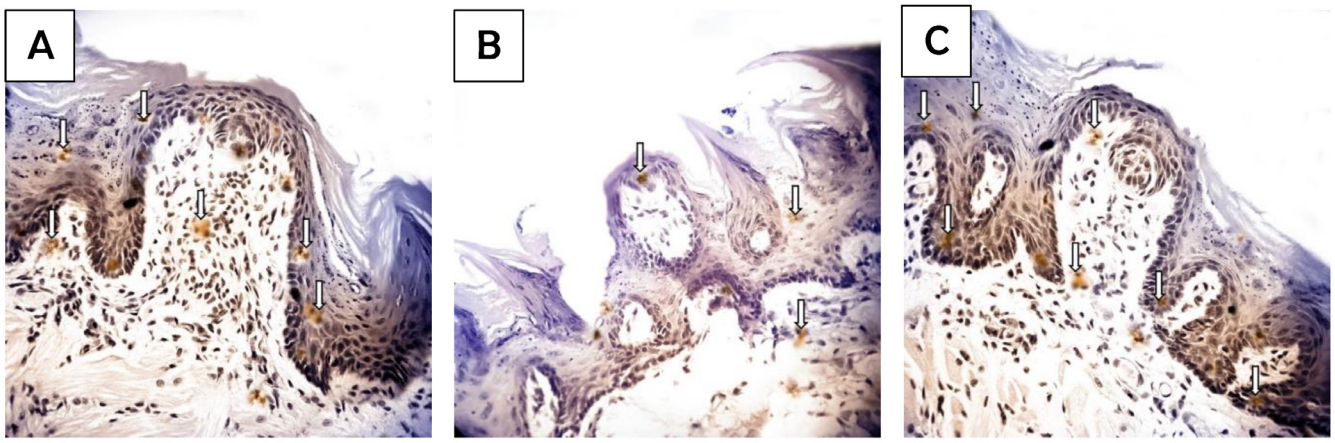

Fig. 5. Photomicrographs of the dorsal surface of the rat tongue, demonstrating the fungiform papillae (B cell lymphoma-2 (Bcl-2), ×400 magnification)

A – group I showing a strong cytoplasmic reaction to Bcl-2 in the basal and suprabasal cell layers, and the underlying connective tissue; B – group II showing a negative cytoplasmic reaction except for few positive cells; C – group III showing a moderate-to-strong cytoplasmic reaction to Bcl-2. White arrows indicate positively stained cells.

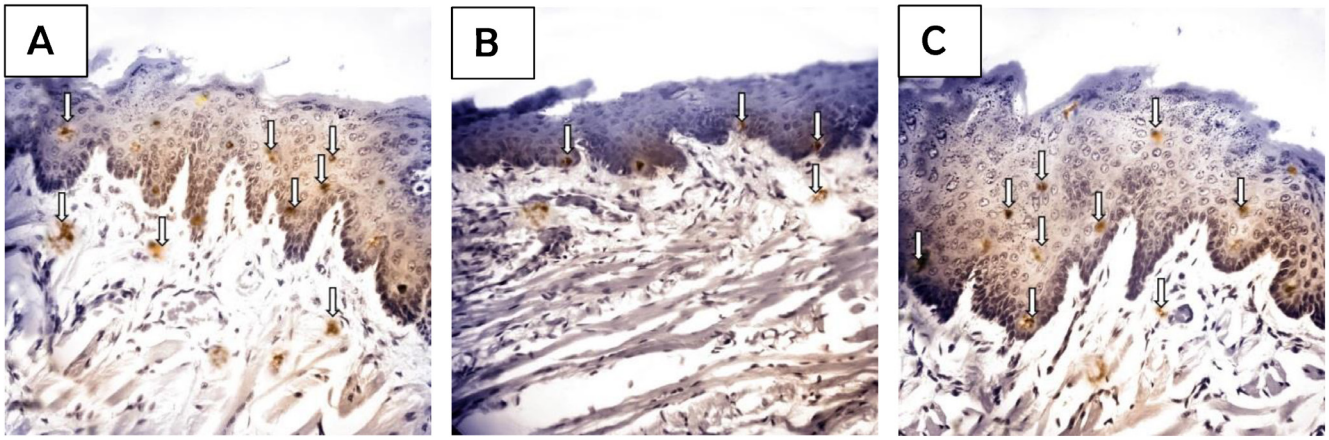

Fig. 6. Photomicrographs of the ventral surface of the rat tongue (B cell lymphoma-2 (Bcl-2), ×400 magnification)

A – group I showing a strong cytoplasmic reaction to Bcl-2 in the epithelial layers and the underlying connective tissue; B – group II showing a weak reaction, mainly in the basal cell layer and the underlying connective tissue; C – group III showing a moderate-to-strong cytoplasmic reaction to Bcl-2 in all epithelial layers and the underlying connective tissue. White arrows indicate positively stained cells.

Table 3. B cell lymphoma-2 (Bcl-2)-stained cell surface area percentage in the filiform and fungiform papillae, and the ventral surface of the tongue in the 3 examined groups

Group	Filiform papillae	Fungiform papillae	Ventral surface of the tongue
I	32.49 ±0.90	32.54 ±1.32	37.18 ±0.52
II	12.24 ±0.96	14.06 ±1.31	24.62 ±0.83
III	31.46 ±0.97	31.14 ±1.31	36.60 ±0.46

Data presented as *M* ±*SD*.

Table 4. Pairwise comparison between the examined groups with regard to the mean B cell lymphoma-2 (Bcl-2)-stained cell surface area percentage in the filiform and fungiform papillae, and the ventral surface of the tongue

Variable	Comparison	*MD*	*p*-value
Filiform papillae	group I vs. group II	20.25	<0.010*
	group I vs. group III	1.03	0.060
	group II vs. group III	19.22	<0.010*
Fungiform papillae	group I vs. group II	18.48	<0.010*
	group I vs. group III	1.40	0.070
	group II vs. group III	17.08	<0.010*
Ventral surface of the tongue	group I vs. group II	12.56	<0.010*
	group I vs. group III	0.58	0.060
	group II vs. group III	11.98	<0.010*

* statistically significant.

The ANOVA displayed statistically significant differences between the 3 groups ($p < 0.01$), with the highest mean Bcl-2-stained cell surface area percentage in the filiform and fungiform papillae, and the ventral surface of the tongue observed in group I (control), and the lowest mean area percentage recorded in group II (TiO_2 NPs).

The pairwise comparison conducted with the use of unpaired Student's *t* test revealed a statistically significant decrease in the mean Bcl-2-stained cell surface area percentage in the filiform and fungiform papillae, and the ventral surface of the tongue in group II (TiO_2 NPs) as compared to both group I (control) and group III (TiO_2 NPs with 4-week recovery) ($p < 0.01$).

Discussion

Titanium dioxide NPs are extensively and frequently utilized in many fields of industry, which increases the risk of exposure and gives rise to serious controversies about their potential effects on the human body. Hence, understanding the effects of TiO_2 NPs on biological systems is essential for the development of safe nanotechnology.

Even though TiO_2 NPs have been considered inert and nontoxic, and are among the most often used NPs, there is increasing evidence suggesting the contrary. Generally, the cytotoxic effects of TiO_2 NPs are accompanied by cell growth inhibition in different cell types.[8,23,24] With the growing use of TiO_2 NPs in the food industry and dentistry, as shown in several studies,[25] there is an urgent need to evaluate the histopathological changes in the lingual mucosa following the administration of TiO_2 NPs in albino rats, as well as possible recovery after their withdrawal.

The TiO_2 administration methods and doses assessed in rats are variable. In this study, the rats received TiO_2 NPs at a dose of 50 mg/kg b.w. per day intragastrically for 30 days as subchronic exposure.[19] The intake of dietary TiO_2 NPs in the United Kingdom has been estimated to be about 5 mg/person/day,[26] which is equivalent to approx. 0.1 mg/kg b.w. per day.[27] In this study, 500 × the estimated human exposure dose (50 mg/kg b.w.) was used as low-dose TiO_2 NP exposure.

Intragastric administration was the route of choice in this study. Previous studies investigated the capacity of TiO_2 NPs to cross biological barriers, such as the gastrointestinal tract. Wang et al. provided evidence that TiO_2 NPs can pass from the gastrointestinal tract through the circulation to the lungs, spleen, liver, and kidneys up to 2 weeks after exposure.[28] Moreover, even when the dose was low, orally administered TiO_2 NPs (5, 50 and 500 mg/kg b.w.) accumulated in mice permanently, which caused inflammation, apoptosis and oxidative stress, and consequently gave rise to chronic gastritis.[29]

The TiO_2 powder with an average size of NPs of 15 ±3 nm, consisting of 95–97% anatase phase and 3–5% brookite phase was used in this study. Weir et al. reported that TiO_2 NPs with a diameter <25 nm in the anatase crystalline phase caused genotoxicity.[30] In comparison with rutile and brookite, anatase has more industrial applications, but it is the most toxic form.[30]

Usually, the shape of the particle plays a key role in the accumulation of TiO_2 NPs in the tissue and cellular response.[31,32] In this study, TiO_2 NPs were spherical in shape. An in vitro study demonstrated a correlation between the shape of TiO_2 NPs and cell penetration.[33] Titanium dioxide NPs penetrated into normal reconstituted human buccal epithelium, with the sphere-shaped NPs found deeper in the epithelium as compared to the spindle-shaped ones.[33] This might be explained by the lower cellular uptake being associated with a high aspect ratio, in which it takes longer for the cell membrane to wrap around the elongated particles.[34]

The light microscopic findings of this study clearly revealed that the administration of TiO_2 NPs caused evident structural changes in the tongue mucosa of the rats (Fig. 1B, 2B and 3B). These changes included atrophied filiform papillae, with an alteration in their normal height and number. They exhibited blunt-ended tops, with a total or partial loss of their characteristic conical shape, and evident hyperkeratosis. Moreover, the fungiform papillae were shrunken and displayed malformed architecture with degenerated taste buds. Similarly, the ventral surface of the tongue displayed atrophied epithelium with an apparent loss of the keratin layer.

This cytotoxic effect of TiO_2 NPs on the tongue mucosa might be mediated by oxidative stress, as reported in an in vitro study.[10] Human hepatocellular cells were exposed to TiO_2 NPs at a concentration of 250 mg/mL, which caused an increase in ROS levels up to two-fold at 5-hour exposure duration, thereby causing DNA damage.[10] An in vivo investigation revealed nuclear shrinkage and chromatin condensation in the nuclei of neurons as well as the DNA ladder in the hippocampus of mice as a result of TiO_2 NP deposition; these features are considered the classical characteristics of apoptosis.[35]

Strikingly, there were areas devoid of the lingual papillae on the dorsal surface of the tongue. This might also be due to a direct effect on the genetic constituents of cells, whereby TiO_2 NPs were able to induce DNA strand breaks and genetic instability.[36] Thus, it could be expected that the widespread use of TiO_2 NPs in many products leads to repeated exposure, which might exhaust the proliferative capability of the epithelium, and ultimately lead to epithelial atrophy.[37]

Occasional inflammatory cell infiltration was observed in the lamina propria of the tongues of the rats exposed to TiO_2 NPs, which might designate the activation of the defense mechanism.[38,39] Parallel results were observed in animals exposed to TiO_2 NPs in other studies; they exhibited inflammation in the tissues of the kidneys and liver.[38,40] Moreover, in the present study, congested blood vessels were noticed (Fig. 3B). This coincides with an in vivo study, which determined that the intraperitoneal injection of TiO_2 NPs created capillary congestion and hemorrhage in the alveolar wall, which resulted from ROS generation.[41]

There was remarkable atrophy and separation in the underlying tongue musculature; this is consistent with the findings of Wang et al., who reported damage in cardiac muscles after 30 days of oral exposure to TiO_2 NPs (0, 10, 50, 200 mg/kg b.w. perday).[42] This muscular damage was further correlated to disrupted levels of proinflammatory cytokines and transcription regulators in the hearts of mice.[43]

In group III (TiO_2 NPs with 4-week recovery), the histological findings (Fig. 1C, 2C and 3C) revealed similar results to those in group I (control) (Fig. 1A, 2A and 3A). The epithelium appeared to have a considerable thickness, with a regular basement membrane, and the underlying lamina propria showed fewer degenerative areas. These results are consistent with those of El-Sheikh et al., who reported moderate improvement in the histopathological changes in rat spleens after the administration of TiO_2 NPs was ceased for 8 weeks.[44]

There was a significant decrease in the mean epithelial thickness in group II (TiO_2 NPs) as compared to both group I and group III ($p < 0.01$ and $p < 0.01$, respectively). The fact that the epithelial thickness was almost back to normal after the recovery period, despite the marked atrophy, indicates notable basal cell proliferation to compensate for this cell loss. Desquamation is one of the most efficient processes by which the stratified squamous epithelium of the body eliminates potentially harmful agents.[45]

The immunohistochemical results showed a statistically significant decrease in the mean Bcl-2 area percentage expressed in group II (TiO_2 NPs) as compared to group I (control) and group III (TiO_2 NPs with 4-week recovery) ($p < 0.01$, $p < 0.01$, respectively). Hu et al. clearly demonstrated that hippocampal apoptosis in response to TiO_2 NP exposure significantly reduced Bcl-2 expression in the hippocampus of mice.[35] Thus, they concluded that TiO_2 NP-induced apoptosis in the mouse hippocampus was primarily mediated through the intrinsic apoptosis pathway.[35]

Conclusions

Our research suggests that a low dose and extended exposure duration, which mimics the potential human exposure to TiO_2 NPs, can lead to cytotoxic effects on the lingual mucosa of albino rats. These cytotoxic effects could be moderately improved by discontinuing the administration of TiO_2 NPs for a period of time. We recommend increasing the period of discontinuation, as complete improvement may occur. In this sense, it is important that people who consume food containing engineered TiO_2 NPs for long periods of time should be particularly concerned. Moreover, our data suggests that the oral cavity should be the subject of TiO_2 NP risk assessment studies in the future.

Ethics approval and consent to participate

The experiment was conducted according to the guidance and approval of the Ethical Committee at the Faculty of Dentistry of Ain Shams University, Cairo, Egypt (approval No.: 615/2017).

Data availability

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

Consent for publication

Not applicable.

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Review

Root resorption factors associated with orthodontic treatment with fixed appliances: A systematic review and meta-analysis

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

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Conflict of interest
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Abstract

External apical root resorption (EARR) is a serious complication that should be avoided during orthodontic treatment; this pathology depends on multiple factors. Data from clinical studies should be assessed to determine the influence these factors have on the development of EARR. This systematic review aims to compare EARR produced by different factors (orthodontic systems, dental trauma, and dental vitality). The protocol was registered on the PROSPERO database. The search was performed on 5 databases. Accepted study designs included randomized controlled trials, nonrandomized clinical trials, and observational studies. Full-text articles from clinical studies of EARR associated with orthodontic treatment in English, Spanish, or Portuguese with no publication date restrictions were selected. Data from the studies, such as age, population, study groups, and outcome measures, were recorded. Multiple meta-analyses were performed with data from the included studies. Evidence suggests that EARR induced by orthodontic treatment is similar, regardless of the technique used. Evidence of the effect of previous dental trauma on EARR during orthodontic treatment is limited. There is less EARR associated with orthodontic treatment in endodontically treated teeth than in vital teeth. These conclusions should be considered with caution due to the low certainty of the evidence.

Keywords: root resorption factors, external apical root resorption, self-ligating brackets, conventional brackets

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Introduction

Orthodontic treatment can lead to complications such as external apical root resorption (EARR),[1] tooth loss,[2] dental fractures,[3] root exposure,[4] demineralization,[5] white spots on the enamel,[6] early closure of the apex,[2] pulpitis,[7,8] periodontal disease,[9] bone resorption,[10] traumatized soft tissues,[11] temporomandibular joint dysfunction,[12] and condylar resorption.[13,14] Individual factors such as short roots and trauma can predispose the patient to these complications.[15,16]

Root resorption (RR) is a serious complication that should be avoided during orthodontic treatment. This pathology depends on multiple factors, including: 1) factors specific to the patient, such as genetics (interleukin (IL)-1β polymorphism),[17] age and gender,[18] personal habits,[2,19] shape of the root,[10] systemic factors,[20] periodontal disease,[21–23] occlusal relationship,[24,25] dental morphology,[26] dental size,[27] traumatized teeth,[28] periapical infection,[29] and previous root resorption[15]; and 2) factors related to orthodontic treatment,[30] such as inadequate biomechanics,[31] long duration of treatment,[32] type of orthodontic movements,[33] intensity of the forces,[34] range of movements, and type of orthodontic appliances.[35] The mandibular and maxillary incisors have been reported as the teeth most susceptible to RR due to orthodontic treatment[36]; therefore, the orthodontist should consider determining factors for proper management during orthodontic treatment.[37] Root resorption can be classified as internal resorption and EARR. The latter is defined as any reduction in the radiographic lengths of the maxillary and mandibular teeth from the tip of the incisal edge or the tip of the most prominent cuspid to the apex of the root. This can be assessed with numerous methods, such as radiography, computed tomography (CT) and the Malmgren root resorption scoring system.[38,39]

This review synthesizes the available evidence regarding EARR; its results will allow orthodontists to make proper clinical decisions in order to minimize the risk of EARR severity. Thus, the aim of this systematic review was to compare EARR due to different conditions: 1) orthodontic systems (self-ligating compared to conventional), 2) dental trauma and 3) dental vitality.

Methods

Protocol and registration

The protocol for this systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) database. It was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[40] and the Cochrane Handbook for Systematic Reviews of Interventions.[41] The PRISMA flow diagrams summarize all of the steps in the selection of included studies and were developed using an online tool.[42]

Eligibility criteria and participant characteristics of the studies

The eligibility criteria for inclusion were defined considering the Participants, Intervention, Comparator, and Outcome (PICO) strategy. The types of studies included in the systematic review were randomized controlled tri-

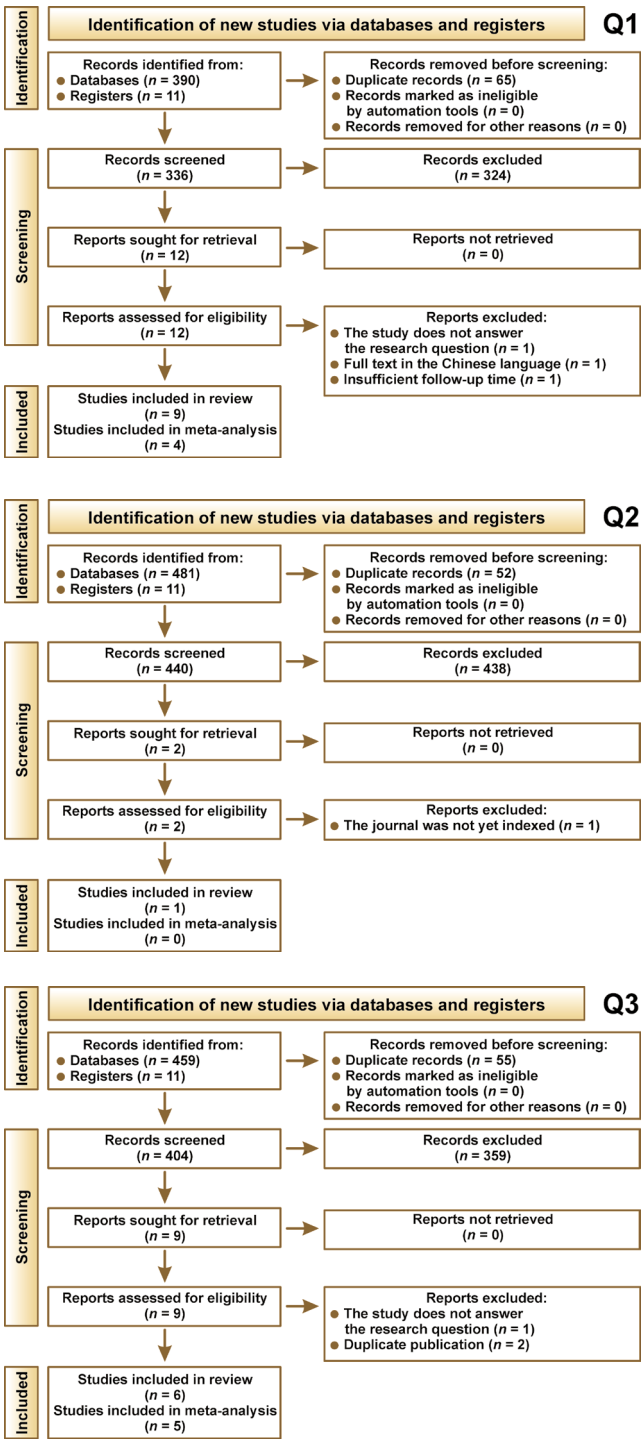


Fig. 1. PRISMA flow diagram search for studies of external apical root resorption (EARR) factors according to each research question

Table 1. Algorithms used in the search strategy adapted for each database and question

PICO strategy	Population: Patients with orthodontic treatment Interventions/condition: Q1 – self-ligating, Q2 – traumatized teeth, Q3 – non-vital teeth Comparator: Q1 – conventional brackets, Q2 – non-traumatized teeth, Q3 – vital teeth Outcomes: Root length, root resorption, root volume, Malmgren score
Focused questions	Q1 – What is the effect on the external apical root resorption induced by orthodontic treatment with self-ligating vs. conventional techniques? Q2 – What is the efffect of dental trauma on external apical root resorption in patients with orthodontic treatment? Q3 – What is the efffect on the external apical root resorption induced by orthodontic treatment in non-vital teeth vs. vital teeth?
Number of registers found for each database	Algorithms used in the search strategy adapted for each database and question
PubMed: Q1 = 7 Q2 = 2 Q3 = 4	Q1 = ("fixed appliances" OR "orthodontic treatment") AND (self-ligating AND (conventional OR "non-self-ligation" OR "traditional brackets")) AND ("apical root resorption" AND "external apical root resorption") Q2 = ("fixed appliances" OR "orthodontic treatment") AND ("traumatized teeth" OR "dental trauma") AND ("apical root resorption" AND "external apical root resorption") Q3 = ("fixed appliances" OR "orthodontic treatment") AND ("root canal treatment" OR "endodontic treatment" OR "non-vital teeth") AND ("apical root resorption" AND "external apical root resorption")
Google Scholar: Q1 = 334 Q2 = 439 Q3 = 395	Q1 = ("fixed appliances" OR "orthodontic treatment") AND ("self-ligating" AND ("conventional" OR "non-self-ligation" OR "traditional brackets")) AND ("apical root resorption" AND "external apical root resorption") Q2 = ("fixed appliances" OR "orthodontic treatment") AND ("traumatized teeth" OR "dental trauma") AND ("apical root resorption" AND "external apical root resorption") Q3 = ("fixed appliances" OR "orthodontic treatment") AND ("root canal treatment" OR "endodontic treatment" OR "non-vital teeth") AND ("apical root resorption" AND "external apical root resorption")
Clinical Trials: 11	orthodontic treatment | root resorption Applied filters: completed
ProQuest: Q1 = 7 Q2 = 2 Q3 = 4	Q1 = ("fixed appliances" OR "orthodontic treatment") AND (self-ligating AND (conventional OR "non-self-ligation" OR "traditional brackets")) AND ("apical root resorption" AND "external apical root resorption") Q2 = ("fixed appliances" OR "orthodontic treatment") AND ("traumatized teeth" OR "dental trauma") AND ("apical root resorption" AND "external apical root resorption") Q3 = ("fixed appliances" OR "orthodontic treatment") AND ("root canal treatment" OR "endodontic treatment" OR "non-vital teeth") AND ("apical root resorption" AND "external apical root resorption")
Web of Science: Q1 = 6 Q2 = 2 Q3 = 4	Q1: TS3 = ("fixed appliances" OR "orthodontic treatment") TS2 = (self-ligating AND (conventional OR "non-self-ligation" OR "traditional brackets")) TS1 = ("apical root resorption" AND "external apical root resorption") Q2: TS = ("fixed appliances" OR "orthodontic treatment") TS = ("traumatized teeth" OR "dental trauma") TS = ("apical root resorption" AND "external apical root resorption") Q3: TS = ("fixed appliances" OR "orthodontic treatment") TS = ("root canal treatment" OR "endodontic treatment" OR "non-vital teeth") TS = ("apical root resorption' AND "external apical root resorption")

als, nonrandomized clinical trials and observational studies. Case reports, case series, letters, comments, short communications, pilot studies (10 patients or less), animal studies, in vitro studies, in silico studies, and literature reviews were excluded. The eligible studies were full-text articles in English, Spanish or Portuguese. There were no publication date restrictions.

Information sources and search strategy

The search was performed on the electronic databases PubMed, ProQuest and Web of Science. The Google Scholar database was used to identify registers and protocols at ClinicalTrials.gov. The manual search was performed through bibliographical references of the studies included in the review (Fig. 1 and Table 1). This search was carried out from July 2019 to October 2019 and updated on June 15, 2021. The keywords and algorithms used for the search strategy are shown in Table 1. Two reviewers (HVS and RTR) performed the search and selection process. In the case of any disagreement, a 3rd reviewer (LAF) resolved the conflict.

Selection process

The studies were evaluated for inclusion in this systematic review by reading the title and abstract of each record identified by the search. The full text of each selected article that matched the eligibility criteria was retrieved for detailed analysis. If the full text did not fully meet the eligibility criteria, those studies were excluded with reasons.

Data collection process and data items

Microsoft Excel (Microsoft Corp., Redmond, USA) was used to generate spreadsheets to record the relevant data of the included studies. These included the demographic characteristics of the participants, groups studies, methodology used, results, baseline measures, follow-up measures, and *p*-values. Two reviewers were responsible for the data extraction (HVS and RTR). In addition, the corresponding authors of the included studies were contacted by email to obtain missing data or additional details.

Risk of bias in individual studies and quality assessment

Two reviewers (HVS and RTR) assessed the risk of bias of the included studies using the Risk of Bias in Non-Randomized Studies of Interventions (Robins-I) tool.[43] Next, the 2 reviewers (HVS and RTR) assessed the quality of the included studies using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach.[44] When there was a disagreement, a 3rd reviewer (LAF) resolved the differences of opinion.

Effect measures and synthesis methods

The main outcome of interest in this review was a decrease in root length related to orthodontic treatment. The effect measure of interest was the mean difference (*MD*) calculated for each study. Qualitative and quantitative syntheses were performed from the data of the included articles. The studies were grouped according to the outcomes that answered the review questions. For the meta-analysis, the information collected from the selected studies was carefully analyzed to determine whether the studies could be pooled. The standardized *MD*s were calculated from the individual studies and combined using a random-effects model. A 95% confidence interval (95% *CI*) and two-sided values were calculated. The heterogeneity between the studies in terms of measures of effect was evaluated using the heterogeneity statistic (I^2), considering that an I^2 value greater than 70% indicated substantial heterogeneity.

Results

Study selection and the results of individual studies

This section was divided according to the answers to Q1, Q2 and Q3. Regarding the archwire sequences used across the studies, the information was provided for some of the studies. Spurrier et al.,[45] Brin et al.,[46] Blake et al.,[47] Mirabella and Årtun,[36] Kreia et al.,[48] Esteves et al.,[49] Llamas-Carreras et al.,[50] Kawashima-Ichinomiya et al.,[51] and Castro et al.[52] did not provide such data. Pandis et al.[53] reported that the conventional group included 0.016 in and 0.020 in copper-nickel-titanium and finished with 0.019 in × 0.025 in stainless steel. In the self-ligating group, the archwire sequence involved a 0.014 in, a 0.016 in × 0.025 in copper-nickel-titanium, and a 0.019 in × 0.025 in stainless steel for finishing. Leite et al. only described that in both groups, the treatments used the same sequence of 0.013 in, 0.014 in, and 0.016 in nickel-titanium archwires; each kind of archwire remained for 2 months.[54] Jacobs et al. used an archwire sequence of a 0.015 in twistflex (stainless steel), 0.016 in nickel-titanium, 0.016 in × 0.022 in nickel-titanium, 0.017 in × 0.025 in nickel-titanium, and 0.019 in × 0.025 in stainless steel in all patients.[55] Chen et al.[39] utilized an initial 0.012 in or 0.014 in nickel-titanium, followed by 0.016 in, 0.018 in, 0.019 in × 0.025 in nickel-titanium, and 0.019 in × 0.025 in stainless steel archwires. For the self-ligating group, Aras et al. used an archwire sequence of 0.014 in copper-nickel-titanium, 0.016 in × 0.025 in copper-nickel-titanium, and 0.019 in × 0.025 in stainless steel, while the control group used 0.016 in copper-nickel-titanium (35°), 0.016 in × 0.022 in copper-nickel-titanium (35°), and 0.019 in × 0.025 in stainless steel archwires.[56] Qin and Zhou followed an archwire sequence that consisted of 0.012 in, 0.016 in, and 0.019 in × 0.025 in copper-nickel-titanium and finished with 0.019 in × 0.025 in stainless steel in the conventional group.[57] The archwire sequence for the self-ligating group included 0.014 in, 0.014 in × 0.025 in copper-nickel-titanium, and finished with 0.019 in × 0.025 in stainless steel.

Q1. EARR associated with orthodontic systems

A total of 390 records of articles were identified in the databases. Sixty-five duplicate records were removed, so 336 records remained, and their titles and abstracts were screened. Twelve articles were retrieved in full text; of these, 3 articles were removed, leaving 9 studies that met all eligibility criteria. The screening process is detailed in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram (Fig. 1), and the synthesis of the results is presented in Table 2.

Except for 2 studies, evaluations of the treatments were carried out at the beginning of the orthodontic treatment and after treatment.[39,47,53,55,57–59] Leite et al. measured EARR before and 6 months after orthodontic treatment initiation.[54] Aras et al. evaluated EARR before and 9 months after the treatment started.[56]

Blake et al. found no statistically significant difference in EARR between self-ligating and conventional appliances.[47] In particular, gender and duration of treatment were not indicators related to EARR. Pan-

dis et al. reported no significant difference between the appliances used.[53] In particular, age, gender, duration of treatment, and dental extractions were not variables related to EARR. Leite et al. found no differences in EARR between the groups studied on the maxillary and mandibular incisors.[54] Kawashima-Ichinomiya et al. reported a statistically significant difference in EARR in favor of self-ligating appliances.[51] In addition, IL-6 levels were evaluated, which were lower in patients treated with self-ligating brackets than conventional brackets. Jacobs et al. reported that there was no difference in EARR between the appliances used.[55] However, using the Malmgren score, they found that the right maxillary central incisors and left maxillary lateral incisors had less EARR (grade 1), while the mandibular central incisors exhibited more EARR (grade 4). Chen et al. also reported no statistically significant differences in EARR using different appliances.[39] Likewise, that study evaluated EARR according to the Malmgren system and found that the self-ligating system group showed a greater distribution of frequencies in grades 1 and 2 compared to conventional appliances. However, the authors did not perform a statistical analysis to determine if there were differences between the grades according to the Malmgren score. Handem et al. found no statistically significant difference between self-ligating and conventional brackets.[59] Aras et al. reported that there were no statistically significant differences in root volume loss between the self-ligating and conventional systems.[56] Qin and Zhou evaluated EARR in patients with class I malocclusion and dental extractions.[57] They found no statistically significant differences in the amount of EARR between the brackets used. Age and gender were not associated with EARR; however, EARR was positively correlated with duration of treatment.

Table 2. Characteristics and results of the included studies of the EARR associated with orthodontic treatment

	Reference	Patients	Groups	Age [years]	Teeth	Assessment method	Results
Q1. Self-ligating vs. conventional techniques	Blake et al. 1995	*n* = 63	GI: conventional fixed appliances, slot 0.018 (Edgewise) (*n* = 33) GII: self-ligating fixed appliances, slot 0.018 (no data) (*n* = 30)	*M* ±*SD* GI: 13 ±2.7 GII:12.10 ±2.3	maxillary lateral incisors maxillary central incisors mandibular lateral incisors mandibular central incisors	panoramic radiograph	EARR *M* ±*SD* maxillary central incisors: GI: 9.41 ±8.63; GII: 7.29 ±6.44 mandibular lateral incisors: GI: 12.83 ±8.65; GII: 12.21 ±9.25 mandibular central incisors: GI: 4.60 ±7.94; GII: 7.36 ±6.86 mandibular lateral incisors: GI: 7.27 ±7.91; GII: 5.00 ±7.63 ($p > 0.05$)
	Pandis et al. 2008	*n* = 96	GI: conventional fixed appliances, slot 0.022 (Microarch) (*n* = 48) GII: self-ligating fixed appliances slot 0.022 (Damon 2) (*n* = 48)	*M* ±*SD* 13.21 ±1.64	maxillary incisors mandibular incisors	panoramic radiograph	EARR *M* ±*SD* maxillary incisors:1.23 ±0.97 mandibular incisors:1.36 ±0.90 univariate model: ($\beta = 0.40$, $SE = 0.26$, p = NS) multivariate model: ($\beta = 0.37$, $SE = 0.20$, $p = 0.06$) ($p > 0.05$)
	Leite et al. 2012	*n* = 19	GI: passive self-ligating fixed appliances, slot 0.022 (Easyclip) (*n* = 11) GII: conventional pre-adjusted fixed appliances, slot 0.022 (3M Unitek) (*n* = 8)	M: 20.6 range: 11–30	maxillary central incisor maxillary lateral incisor mandibular central incisor mandibular lateral incisor	CBCT	EARR *M* ±*SD* maxillary central incisor: GI: −0.34 ±0.24; GII: −0.33 ±0.19 maxillary lateral incisor: GI: −0.43 ±0.33; GII: −0.44 ±0.33 mandibular central incisor: GI: −0.39 ±0.52; GII: −0.31 ±0.21 mandibular lateral incisor: GI: −0.23 ±0.23; GII: −0.40 ±0.24 ($p > 0.05$)
	Kawashima-Ichinomiya et al. 2012	*n* = 60	GI: passive self-ligating fixed appliances, slot no data (Damon) (*n* = 30) GII: conventional fixed appliances, slot 0.022 (Edgewise) (*n* = 30)	*M* ±*SD* 18 ±5.3	maxillary central incisor	periapical radiographs and cephalograms	EARR *M* ±*SD* maxillary central incisor: GI 2.5 ±1.5; GII: 0.88 ±0.9 ($p = 0.005$)
	Jacobs et al. 2014	*n* = 213	GI: self-ligating fixed appliances, slot 0.022 (Smart clip) (*n* = 139) GII: conventional fixed appliances, slot 0.022 (Victory) (*n* = 74)	*M* ±*SD* 12.4 ±2.2	maxillary and mandibular incisors	panoramic radiograph	rRR *M* ±*SD* GI: 3.0 ±5.6; GII: 4.5 ±6.6 sEARR % GI: *n* = 3–0.3%; GII: *n* = 3–0.5% ($p = 0.33$)

	Reference	Patients	Groups	Age [years]	Teeth	Assessment method	Results
Q1. Self-ligating vs. conventional techniques	Chen et al. 2015	*n* = 70	GI: passive self-ligating fixed appliances, slot 0.022 (Damon 3) (*n* = 35) GII: conventional fixed appliances, slot 0.022 (3M Unitek) (*n* = 35)	*M* ±*SD* GI:13.52 ±2.84 GII:13.42 ±2.50	maxillary central incisor maxillary lateral incisor mandibular central incisor mandibular lateral incisor	periapical radiograph	EARR *M* ±*SD* maxillary central incisor: GI: 0.3 ±0.4; GII: 0.5 ±0.3 maxillary lateral incisor: GI: 0.2 ±0.3; GII: 0.3 ±0.5 mandibular central incisor: GI: 0.4 ±0.4; GII: 0.4 ±0.5 mandibular lateral incisor: GI: 0.3 ±0.3; GII: 0.3 ±0.5 ($p > 0.05$) Malmgren score: GI: *n* = 280 (%) score 0: 0 (0%) score 1: 188 (67.14%) score 2: 67 (23.93%) score 3: 22 (7.86%) score 4: 3 (1.07%) GII: *n* = 280 (%) score 0: 0 (0%) score 1: 156 (55.71%) score 2: 70 (25.00%) score 3: 48 (17.14%) score 4: 6 (2.14%) teeth *n* = 560 (%) score 0: 0 (0%) score 1: 344 (61.4%) score 2: 137 (24.4%) score 3: 70 (12.5%) score 4: 9 (1.6%) (p = ND)
	Handem et al. 2016	*n* = 52	GI: self-ligating fixed appliances, slot 0.022 (Damon) (*n* = 25) GII: conventional fixed appliances, slot 0.022 (Roth) (*n* = 27)	range: GI:16.04–18.06 GII:16.77–18.47	maxillary central incisor maxillary lateral incisor mandibular central incisor mandibular lateral incisor	periapical radiograph	EARR M [±*SD*?] maxillary right central incisor: GI: 0.72; GII: 0.59 maxillary right lateral incisor: GI: 0.72; GII: 0.70 maxillary left central incisor: GI: 0.88; GII: 0.66 maxillary left lateral incisor: GI: 0.80; GII: 0.74 mandibular right central incisor: GI: 0.64; GII: 0.66 mandibular right lateral incisor: GI: 0.48; GII: 0.40 mandibular left central incisor: GI: 0.60; GII: 0.62 mandibular left lateral incisor: GI: 0.56; GII: 0.55 ($p > 0.05$) Malmgren score: GI: *n* = 200 (%) score 0: 93 (46.5%) score 1: 83 (41.5%) score 2: 20 (10%) score 3: 4 (2%) score 4: 0 (0%) GII: *n* = 216 (%) score 0: 114 (52.7%) score 1: 74 (34.2%) score 2: 24 (11.1%) score 3: 4 (1.85%) score 4: 0 (0%) teeth *n* = 560 (%) score 0: 207 (49.7%) score 1: 157 (37.7%) score 2: 44 (10.5%) score 3: 8 (1.92%) score 4: 0 (0%) (p = ND)

	Reference	Patients	Groups	Age [years]	Teeth	Assessment method	Results
Q1. Self-ligating vs. conventional techniques	Aras et al. 2018	*n* = 32	GI: self-ligating fixed appliances, slot 0.022 (Damon Q) (*n* = 16) GII: conventional fixed appliances, slot 0.022 (Titanium Orthos) (*n* = 16)	*M* ±*SD*: GI:15.00 ±1.03 GII:14.94 ±1.06	central incisor lateral incisor	CBCT	volumetric changes *M* ±*SD* central incisor: GI: 27.08 ±12.71; GII: 28.29 ±13.48 intergroup differences: (*p* = 0.712) lateral incisor: GI: 20.32 ±11.67; GII: 19.77 ±11.05 intergroup differences: (*p* = 0.587)
	Qin and Zhou 2019	*n* = 98	GI: self-ligating fixed appliances, slot 0.019 (Damon 3) (*n* = 49) GII: conventional fixed appliances, slot 0.019 (3M Unitek) (*n* = 49)	*M* ±*SD*: GI: 15.21 ±4.43 GII:15.15 ±4.52	maxillary central incisor maxillary lateral incisor mandibular central incisor mandibular lateral incisor	panoramic radiograph	EARR *M* ±*SD* maxillary right central incisor: GI: −0.32 ±0.24; GII: −0.40 ±0.28 maxillary right lateral incisor: GI: −0.27 ±0.28; GII: −0.30 ±0.25 maxillary left central incisor: GI: −0.33 ±0.27; GII: −0.39 ±0.31 maxillary left lateral incisor: GI: −0.28 ±0.25; GII: −0.31 ±0.26 (*p* > 0.05)
Q2. Dental trauma vs. non-dental trauma	Brin et al. 1991	*n* = 139	group T: 'trauma' group (*n* = 56) group O: 'orthodontic' group (*n* = 29) group TO: 'trauma-orthodontic' group (*n* = 28) group C: 'intact' control group (*n* = 26)	M: 13.7	maxillary incisors	periapical radiograph	prevalence of root resorption (%): group T: 7.8% group O: 6.7% group TO: 27.8% group C: 0.0%
Q3. Non-vital vs. vital teeth	Spurrier et al. 1990	*n* = 43	split-mouth GI: RFT GII: VHT	range: 13–11	incisors	periapical radiographs	EARR *M* ±*SD* RFT: 1.28 ±1.09 VHT: 2.05 ±1.49 (*p* = 0.006)
	Mirabella and Årtun 1995	*n* = 36	split-mouth GI: RFT GII: VHT	range: 20–70	anterior maxillary teeth	periapical radiographs and cephalograms	The mean difference in root resorption between the contralateral pairs of teeth (*n* = 39) with and without endodontic treatment was 0.45 ±1.21 (*p* < 0.05).
	Kreia et al. 2005	*n* = 40	split-mouth GI: RFT GII: VHT	ND	maxillary incisors	periapical radiographs	EARR *M* ±*SD* RFT: 1.14 ±1.03 VHT: 1.34 ±1.35 (*p* = 0.903)
	Esteves et al. 2007	*n* = 16	split-mouth GI: RFT GII: VHT	ND	maxillary incisors	periapical radiographs	EARR *M* ±*SD* RFT: 0.82 ±1.19 VHT: 1.04 ±1 (*p* = 0.29)
	Llamas-Carreras et al. 2012	*n* = 38	split-mouth GI: RFT GII: VHT	*M* ±*SD* 31 ±10	maxillary incisors	digital panoramic radiographs	EARR *M* ±*SD* RFT: 1.1 ±0.8 VHT: 1.1 ±1.0 PRR: 1.0 ±0.2 (*p* > 0.05)
	Castro et al. 2015	*n* = 20	split-mouth GI: RFT GII: VHT	range: 11–15	posterior teeth	CBCT	tooth length *M* ±*SD* RFT before orthodontic treatment: 20.55 ±1.21 after orthodontic treatment: 20.25 ±1.18 VHT before orthodontic treatment: 20.29 ±1.33 after orthodontic treatment: 20.13 ±1.57 (*p* = 0.4197)

GI – group I; GII – group II; rRR – relative root resorption; EARR – external apical root resorption; sEARR – severe external apical root resorption; *M* – mean; *M* ±*SD* – mean ± standard deviation; 95% *CI* – 95% confidence interval; *OR* – odds ratio; *SE* – standard error; ND – no data; NS – not statistically significant; CBCT – cone-beam computed tomography; RFT – root filled tooth; VHT – vital homologous teeth; PRR – proportion of root resorption.

Risk of bias and quality assessment

The studies included in this review showed moderate risk of bias. The main deficiencies were found in the domains Confounding (100%), Missing data (55%), Deviations from interventions (11%), and Measuring outcomes (11%). The quality of the articles was low to moderate, with a critical level of evidence. Risk of bias and the results of quality assessments are shown in Fig.2 and Tables 3–5, respectively.

Meta-analysis

The heterogeneity found in the meta-analysis of EARR of maxillary central incisor teeth was very high (I^2 = 89%). No statistically significant difference was found between the EARR of teeth orthodontically treated with self-ligating and conventional techniques (p = 0.76). When analyzing the variations between the results of the studies, it was found that there were differences in both the age and sex of the participants that could have influenced the effect of the intervention. The age of the participants varied considerably. In 2 of the studies,[51,54] the mean age was 18.0 ±5.3 years and 20.6 years, while in the other 2 studies,[39,57] the mean age was 13.52 ±2.84 years and 15 years. Thus, it is apparent that the participants in the first 2 studies were young adults, while the last 2 studies evaluated adolescent participants. Additionally, the female:male ratio in 1 study was 3:1,[51] while it was 1:1 in the other

Table 3. Quality assessment of the included studies that evaluated EARR associated with orthodontic treatment (Q1. Self-ligating vs. conventional appliances)

	Certainty assessment						Number of patients		Effect		Certainty	Importance
	Number of studies	study design	risk of bias	inconsistency	indirectness	imprecision	NO-SL	SL	relative (95% *CI*)	absolute (95% *CI*)		
Maxillary central incisors	4	observational studies	serious	serious	not serious	not serious	106	106	–	MD 0.3 lower (0.86 lower to 0.26 higher)	⊕⊕○○ low	critical
Maxillary lateral incisors	3	observational studies	serious	not serious	not serious	not serious	76	76	–	MD 0.07 higher (0.09 lower to 0.24 higher)	⊕⊕⊕○ moderate	critical
Mandibular central incisors	3	observational studies	serious	not serious	not serious	not serious	76	76	–	MD 0.04 lower (0.25 lower to 0.18 higher)	⊕⊕⊕○ moderate	critical
Mandibular lateral incisors	3	observational studies	serious	not serious	not serious	not serious	76	76	–	MD 0.08 higher (0.1 lower to 0.27 higher)	⊕⊕⊕○ moderate	critical

95% *CI* – 95% confidence interval; *MD* – mean difference.

Table 4. Quality assessment of the included studies that evaluated EARR associated with orthodontic treatment (Q2. Dental trauma vs. non-dental trauma)

Number of studies	Results	Impact	Number of participants	Certainty	Importance
1	Reabsorption from dental trauma. Evaluated using periapical radiographs. Follow-up: Half a year after the end of the retention period.	Reabsorption was more frequent indirectly injured teeth than in the group with dental trauma before orthodontic treatment. No further information.	139	⊕○○○ very low	critical

95% *CI* – 95% confidence interval; *MD* – mean difference.

Table 5. Quality assessment of the included studies that evaluated EARR associated with orthodontic treatment (Q1. Self-ligating vs. conventional appliances)

	Certainty assessment						Number of patients		Effect		Certainty	Importance
	Number of studies	study design	risk of bias	inconsistency	indirectness	imprecision	Endodontically treated	Vital teeth	relative % (95% *CI*)	absolute % (95% *CI*)		
Non-vital vs. vital incisor teeth	5	observational studies	serious	serious	not serious	not serious	145	145	–	MD 0.28 lower (0.51 lower to 0.05 lower)	⊕⊕○○ low	critical
Non-vital vs. vital posterior teeth	1	observational studies	serious	serious	not serious	not serious	20	20	–	MD 0.2 higher (0.42 lower to 0.82 higher)	⊕⊕⊕○ moderate	critical

95% *CI* – 95% confidence interval; *MD* – mean difference.

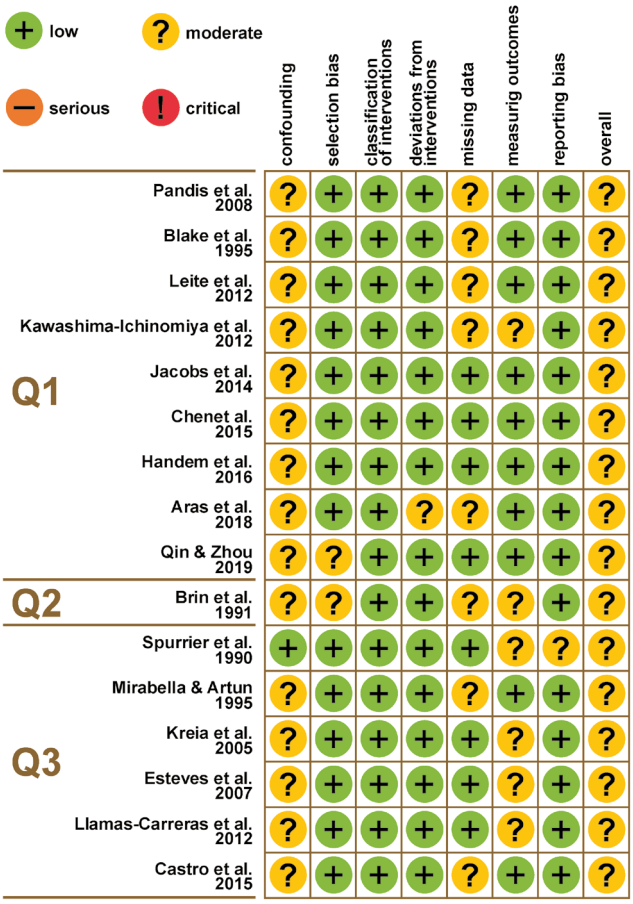


Fig. 2. Risk of bias in the included studies that assessed EARR factors according to each research question

3 studies.[39,54,57] Although the 4 studies included in this meta-analysis exhibited moderate risk of bias, 1 presented more domains with risk of bias[51] compared to the other studies (Fig. 3). In the meta-analysis of the EARR of the maxillary lateral incisor teeth, there was no statistically significant difference (Fig. 3). In the meta-analysis of the EARR of the mandibular central incisor teeth, there was no statistically significant difference between the appliances (Fig. 3). There was no heterogeneity in either analysis. In the meta-analysis of the EARR of the mandibular lateral incisor teeth, it was found that there was no statistically significant difference (p = 0.49); moderate heterogeneity was demonstrated (Fig. 3).

Q2. EARR associated with dental trauma

The search identified a total of 481 articles. Fifty-two duplicate records were removed, so the titles and abstracts of 440 records that remained were screened. Two articles were retrieved in full text; 1 article was removed,[38] resulting in 1 study that met all of the eligibility criteria. The screening process is detailed in the PRISMA flow diagram (Fig. 1), and the synthesis of the results is presented in Table 2.

Brin et al. evaluated the EARR of traumatized permanent maxillary incisors which underwent orthodontic treatment using periapical radiographs, with a 6-month follow-up after completion of the retention period.[46] That study examined 4 groups: with dental trauma, with orthodontic treatment, with trauma and orthodontic treatment, and the control group without trauma or orthodontic treatment. The results suggest that the combination of dental trauma and orthodontic treatment results in teeth that are more susceptible to RR. This study evaluated EARR linked to treatment duration without finding any correlation.

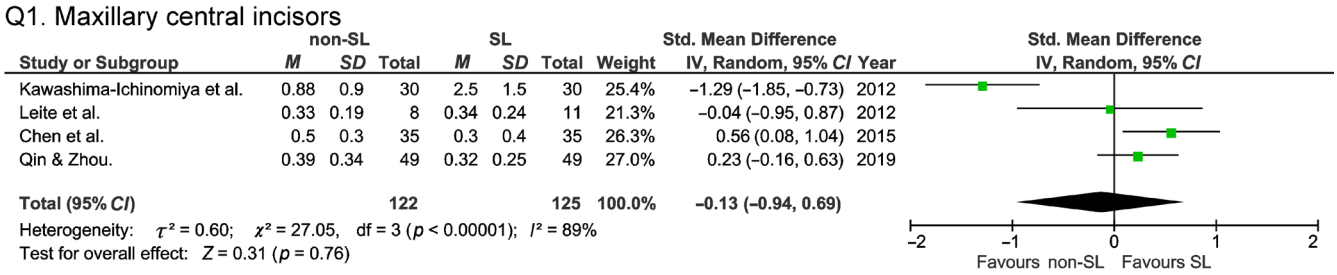
Risk of bias and quality assessment

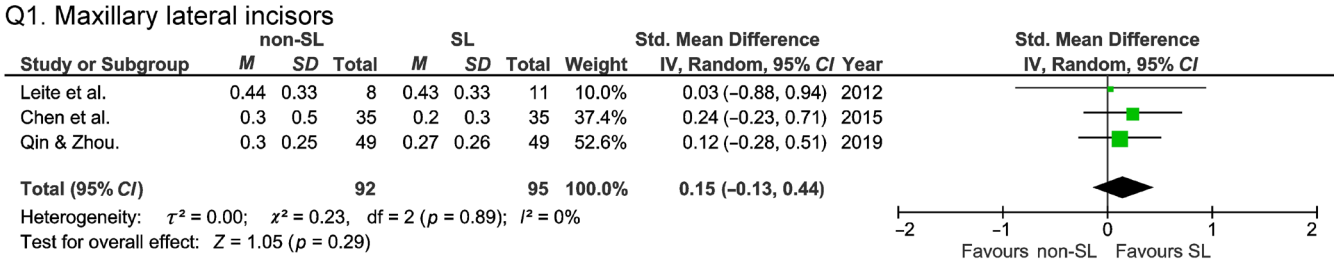
The included study showed moderate risk of bias; the main deficiencies were found in the Confounding, Selection bias, Missing data, and Measuring outcome domains. In the quality assessment, very low certainty of the evidence was observed due to 1) risk of bias, 2) non-assessable consistency between findings in the literature for a single study on the effect of trauma on RAS during orthodontic treatment, and 3) indirection due to the evaluation of a surrogate variable. Risk of bias and quality assessments are shown in Fig. 2 and Tables 3–5, respectively.

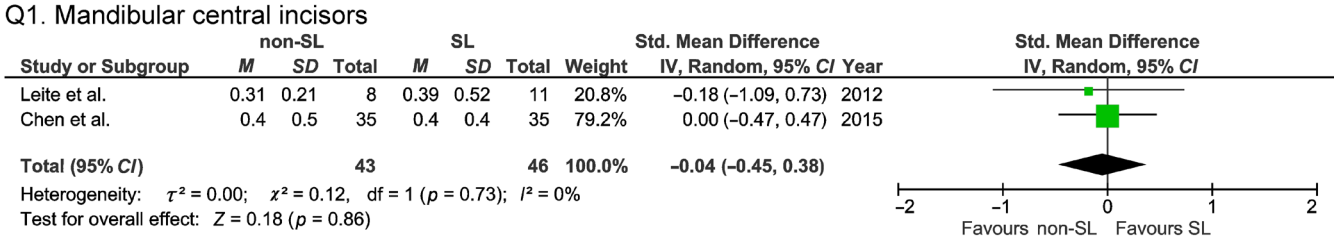
Q3. EARR associated with dental vitality

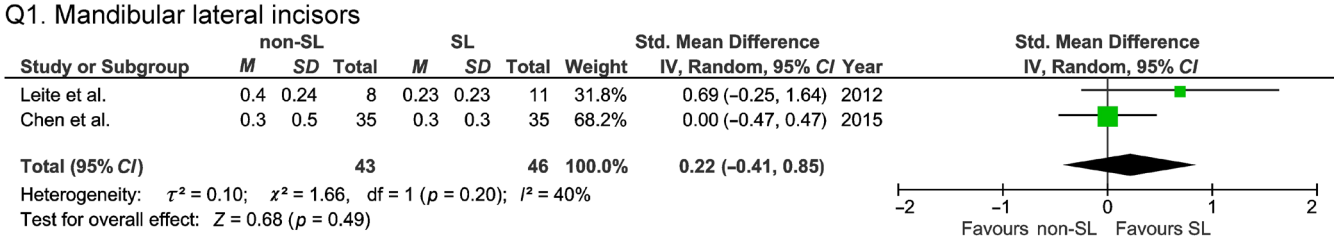
The search identified a total of 459 records of articles. Fifty-five duplicate records were removed, so 404 records remained, and their titles and abstracts were screened. Nine articles were retrieved in full text; of these, 3 articles were removed,[60–62] resulting in 6 studies[36,45,48–50,52] that met all of the eligibility criteria; there were 173 participants in total. Mirabella and Årtun[36,60] reported the results from 2 articles, and Llamas-Carreras et al.[50,61] reported results from the same sample. Thus, this review included only 1 study by Mirabella and Årtun[36] and 1 study by Llamas-Carreras et al.[50] which provided the necessary data for answering the review question. The screening process is detailed in the PRISMA flow diagram (Fig. 1), and the synthesis of the results is shown in Table 2.

The measurements to determine the amount of EARR in all of the studies were performed before and after orthodontic treatment and had a split-mouth design. Corresponding vital homologous teeth were used as controls for comparison. Spurrier et al.[45] demonstrated that vital homologous teeth suffered more resorption than endodontically treated teeth; this difference was statistically significant. This study assessed EARR linked to gender and found no correlation. Mirabella and Årtun,[36] Kreia et al.,[48] Esteves et al.,[49] Llamas-Carreras et al.,[50] and Castro et al.[52] reported that non-vital teeth exhibited similar resorption to their contralateral vital teeth.









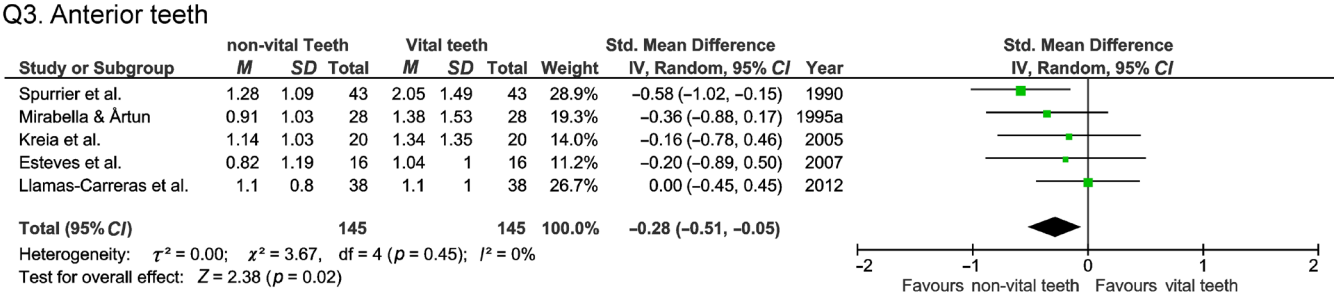


Fig. 3. Forrest plots of EARR on anterior teeth using self-ligation (SL) compare to conventional (Non-SL) appliances and non-vital compared to vital teeth

Risk of bias and quality assessment

The included studies showed moderate risk of bias; the main deficiencies were found in the Confounding (85.7%), Missing data (42.8%), Measuring outcomes (42.8%), and Reporting bias (14%) domains. The quality of the studies was low, with a critical level of evidence. The risk of bias and quality assessments are shown in Fig. 2 and Tables 3–5, respectively.

Meta-analysis

The sample analyzed by Mirabella and Årtun[36] consisted of 36 patients with 39 endodontically treated and their vital homologous anterior teeth, which included the canines. However, for the meta-analysis, the remaining studies used the incisors only. As a result, a subgroup from the sample was used following the same strategy as previously reported in a systematic review by Ioannidou-Marathiotou et al.[63] The study that evaluated the amount of EARR in molar teeth was not included in the meta-analysis due to the type of teeth studied.[52] The data from 145 patients was pooled in the meta-analysis of EARR for non-vital and vital teeth.[36,45,48–50] It was determined that there was a statistically significant difference between the groups; i.e., the non-vital teeth showed less EARR compared to their contralateral vital teeth following orthodontic treatment (Fig. 3).

Discussion

Q1. EARR produced by the self-ligating compared to conventional techniques

Analysis of the articles included in this systematic review suggested that there is no statistically significant difference in EARR when comparing self-ligating and conventional techniques. However, 9 observational articles were analyzed that had moderate risk of bias and moderate quality of evidence. No controlled clinical trials were found; therefore, the studies provide limited evidence.

A review performed by Yi et al. evaluated studies of EARR due to orthodontic treatment with self-ligating and conventional techniques.[64] They assessed 7 studies, 5 of which were pooled for a meta-analysis.[39,47,54,65,66] Their results suggest that the use of self-ligating brackets causes less EARR in maxillary central incisor teeth during orthodontic treatment (without differences in the rest of the teeth evaluated). However, the studies included in that review had moderate risk of bias, and no quality assessment was performed. Their heterogeneity results ($I^2 = 0$) did not correlate with the confidence intervals and direction of effect from the included studies. Finally, concerning the maxillary lateral incisors, mandibular central incisors and mandibular lateral incisors, there was no statistically significant difference in the amount of EARR between the 2 techniques.

Q2. EARR of traumatized compared to non-traumatized teeth

Resorption was more frequent in directly injured teeth in the group with dental trauma before orthodontic treatment; however, that study was observational and had moderate risk of bias and very low quality. No controlled clinical trials were found. Consequently, the evidence is limited, so more studies are required to determine whether there is a direct relationship between previous dental trauma and EARR in patients undergoing orthodontic treatment. In the same sense, no systematic reviews of this topic were found.

Q3. EARR of non-vital compared to vital teeth

The included studies suggest that there is a statistically significant difference in the amount of EARR in endodontically treated teeth compared to homologous vital teeth after orthodontic treatment. In this systematic review, 6 observational studies were analyzed; 5 of those included in the meta-analysis showed low quality.[36,45,48–50] When conducting the bias assessment, these studies were determined to have moderate risk of bias. No controlled clinical trials were found. Consequently, the evidence is limited regarding the effect of orthodontic treatment on the amount of EARR in non-vital compared to vital teeth.

The systematic review carried out by Ioannidou-Marathiotou et al.[63] assessed 6 studies[36,45,48,49,60,61] that focused on the amount of EARR in endodontically treated teeth after orthodontic treatment. They reported that endodontically treated teeth exhibited relatively less RR than teeth with vital pulps. Their meta-analysis included 4 articles[36,45,48,49] and revealed a statistically significant difference ($p = 0.005$) between the groups and low heterogeneity ($I^2 = 0\%$) across the studies. In the review by this research team, no evaluation of bias was performed. In addition, the overall quality of the included studies was considered low.

Currently, there are no specific and effective treatments for EARR. Nanotechnology has been used as a novel approach for dentistry treatments[67–70]; in that sense, chitosan and hydroxyapatite nanoparticles have been developed for the treatment of EARR. Unfortunately, the studies that have been carried out so far have been animal models and case reports.[71,72] Consequently, the only proven available management of EARR is preventive through adequate diagnosis and orthodontic treatment.

On the current market, clear aligner therapy (CAT) has become a popular option when compared to fixed appliances due to its advantages of superior esthetics and comfort.[73] Aldeeri et al. performed a systematic review that aimed to evaluate the evidence concerning EARR in CAT during orthodontic treatment.[74] They reported a low risk of EARR associated with CAT. However, due to the design and quality of the included studies, solid evidence could not be established. Later, Fang et al. performed a meta-analysis aimed at assessing the amount of EARR in patients undergoing orthodontic treatment with CAT compared to those treated with fixed appliances.[75] They reported that the incidence and severity of EARR were

lower in those using CAT. Finally, Gandhi et al. carried out a meta-analysis that aimed to investigate EARR in patients treated with pre-adjusted edgewise appliances compared to those treated with CAT.[76] They concluded that neither pre-adjusted edgewise appliances nor CAT resulted in clinically significant EARR. However, the amount of EARR was higher in the pre-adjusted edgewise appliance users only in tooth 12. We can conclude that the use of CAT could diminish the risk of EARR. However, there is a lack of satisfactory quality of evidence in the studies included in the previously mentioned reviews.

At present, the number of publications in basic science, observational studies and clinical trials has been increasing exponentially. However, randomized clinical trials are considered the best evidence to evaluate a health problem. The literature includes mixed reports of basic science results that are not automatically reflected in clinical practice.[77,78] Moreover, several publications in dentistry report divergent results despite presenting characteristics that superficially seem similar or use different variables to determine the effect of an intervention.[79,80] Hence the importance of evidence-based medicine aimed to determine the validity and analyze the data set of published studies through systematic reviews.

Limitations

This review encountered several limitations, including limited research in the field, risk of bias, low quality of the observational studies, and lack of randomized clinical trials that met the inclusion criteria.

Conclusions

The evidence suggests that the EARR induced by orthodontic treatment with self-ligating or conventional brackets is similar, regardless of the technique used. However, although the meta-analysis had low heterogeneity, the included studies exhibited a moderate risk of bias and low to moderate quality. Future studies are required with adequate internal and external validity, and EARR assessment methods used in the future should be more accurate.

The evidence on the effect of dental trauma on EARR during orthodontic treatment is limited, with very low quality and moderate risk of bias in the studies assessed. There are no conclusive results from this analysis.

The current evidence suggests that the amount of EARR during orthodontic treatment in endodontically treated teeth is lower than in vital teeth. However, the included studies showed a moderate risk of bias and low quality.

Ethics approval and consent to participate

Not applicaple.

Data availability

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

Consent for publication

Not applicable.

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Review

Successful management of dentin hypersensitivity: A narrative review

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Abstract

The prevalence of dentin hypersensitivity (DH) is increasing around the world. At least one in 10 individuals in the general population has been diagnosed with DH. It is a diagnosis that has significant negative effects on a person's oral health-related quality of life. This condition, which is characterized by sharp, short tooth pain in response to thermal, chemical, tactile, and evaporative stimuli, is more commonly seen in adults. DH has a tremendous impact on the social and financial aspects of patients and society at large. It is essential to recognize the factors that can contribute to a successful treatment outcome to guarantee the overall well-being of DH patients.

The aim of this narrative review was to highlight strategies that can lead to successful DH treatment outcomes, along with current updates on DH mechanisms, treatment options, and the latest management approaches. A positive treatment outcome for DH requires a concerted effort from both the patient and the dental practitioner. Highly motivated patients and dental practitioners with sound knowledge of DH diagnosis and available treatment options will ensure successful long-term improvement of DH symptoms.

Keywords: therapy, treatment outcome, pain, dentin hypersensitivity

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Introduction

Tooth sensitivity, which is clinically referred to as dentin hypersensitivity (DH), is becoming increasingly more common in dental practice.[1–3] DH is described as short, sharp pain arising from the teeth in response to hot and cold temperatures and evaporative, tactile, osmotic, and chemical stimuli. It cannot be attributed to any dental defects or disease.[4] At least one in 10 individuals in the general population suffers from DH, which causes discomfort and pain and compromises quality of life.[3,5–7] Reports of DH prevalence range from as low as 1.3% to as high as 92.1%, depending on sociodemographic characteristics, recruitment strategies, and number of study sites involved.[1] DH is more prevalent in adults and imposes significant impacts on social and financial aspects for patients and society at large.[1,8,9] Understanding DH and identifying the factors that contribute to successful treatment outcomes are essential to ensure not only improvement of DH patients' oral health condition but their overall well-being. Thus, the aim of this narrative review was to highlight strategies for successful DH treatment outcomes, along with current updates on DH mechanisms, treatment options, and the latest management protocols.

Material and methods

A computerized literature search on DH management was performed using the PubMed and Google Scholar databases. The keywords searched included "dentin hypersensitivity management", "dentin hypersensitivity treatment", "dentin hypersensitivity prevention", "dental pain", "therapy for dentin hypersensitivity", and "dentin hypersensitivity and treatment outcome". The articles included in this review were from 2011 to 2021. Moreover, some earlier references were also cited according to their relevance to current research progress on the management of DH. All relevant original articles, review articles, and case reports available in full text and published in English were included.

Pathophysiology and mechanisms of pain in dentin hypersensitivity

Dentin is the middle layer of the tooth that makes up the bulk of a tooth's structure. It is composed of closely packed dentinal tubules that are aligned throughout its thickness and contain odontoblastic processes. The odontoblast cell bodies that synthesize dentin are aligned at the inner dentinal layer, which indirectly forms the boundary of the dentin-pulp complex. Under normal conditions, dentin is protected from the external oral environment by the outermost mineralized enamel layer (for coronal dentin) or cementum (for cervical dentin). The innermost layer is composed of pulpal tissue and is where the primary dental afferents are found. Dentin loses its protection when the enamel layer disappears or when gingival tissue recedes, exposing the dentin layer and the dentinal tubules within. These structural changes can result from acidic food intake, overzealous dental hygiene practices, and parafunctional habits.[2,10] The discomfort and pain associated with DH develop as a result of these activities; however, as pain signaling requires stimulation of the free nerve endings on the primary afferent neurons and that dentin is lacking neuronal fibers,[11] the exact mechanism to explain this common intraoral condition remains elusive.

Three theories have been proposed to explain the mechanism of pain in DH: the hydrodynamic, direct innervation, and odontoblast transducer theories (Fig. 1). The hydrodynamic theory proposed by Braennstroem and Astroem[12] remains the most widely accepted mechanism.

The neural theory was among the earliest DH theories brought forward. Indeed, the involvement of nerve fibers in pain signaling is an ideal hypothesis to explain DH, as it has already served as a well-established explanation for pain mechanisms in other parts of the body. However, there are conflicting reports about the presence of nerve fibers in dentin and dentinal tubules; more studies support the absence of nerve fibers within dentin and dentinal tubules.[11,13,14] In this regard, state-of-the-art 3D imaging techniques – CLARITY and light sheet microscopy – have enabled imaging of intact tissues with high resolution and preserved anatomical structures.[14] Using fluorescent dyes, the initial findings of this technique revealed pronounced nerve bundles and their branches running along the longitudinal axis of the root from the apical foramina toward the coronal pulp with terminal endings close to the dental pulp periphery.[14] However, as this study only involved imaging of the pulp tissue, extension of the peripheral terminals (whether or not they reach the

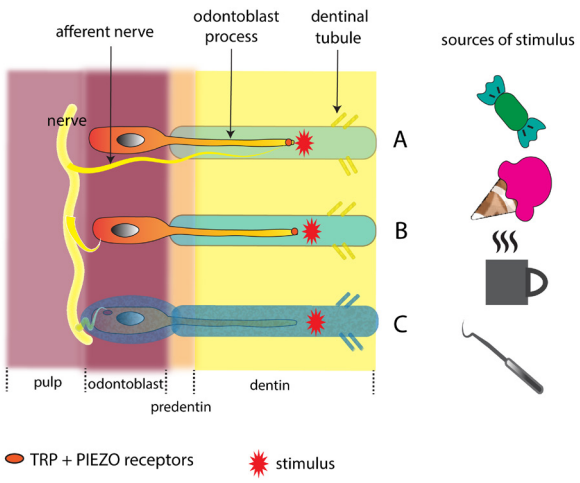

Fig. 1. Scheme for dentin hypersensitivity

periphery of the dentin) has yet to be determined. Future studies using CLARITY and light sheet microscopy that preserve the dentin-pulp complex could resolve the conflicting findings on the extension and presence of nerve terminals into the dentin.

Another theory, the odontoblast transducer theory, was proposed by Rapp et al.[15] They hypothesized that odontoblasts act as sensory cells that are triggered by increased intratubular fluid movement, transducing this movement into electrical signals and synapsing with nearby primary dental afferent nerve endings at the dentin-pulp junction. This posited role of odontoblasts as sensory cells probably stems from their embryogenic origin; they are derived from the ectomesenchyme cells of the neural crest,[16] similar to neurons.[17] Recent research has revealed that mechanosensitive and thermosensitive receptor channels (Piezo channels) and several members of the transient receptor potential superfamily ion channels are expressed in odontoblasts,[18,19] which further supports the odontoblast transducer theory.

The concept of the hydrodynamic theory was proposed by Gysi, when he explained that dentinal pain is associated with fluid movement in the dentinal tubules.[20] However, it was only in the 1960s that Braennstroem and Astroem were able to demonstrate that when mechanical stimulation, cold and hot temperatures, and osmotic pressure (caused by sugar or salt intake) were introduced at the site of exposed dentin, there was an increase in fluid movement into the dentinal tubules; this led to DH.[12] Brännström proposed this mechanism as the hydrodynamic theory.[21] Further studies have demonstrated that this increased fluid movement into the dentinal tubules excites both C- and Aδ-fibers in the dentin–pulp junction, which in turn activates the dental pain signaling pathway.[22,23] The expression of thermosensitive and mechanosensitive receptors, as well as voltage-gated ion channels involved in pain signaling in the primary dental afferent neurons,[19] further supports the hydrodynamic theory.

Management of dentin hypersensitivity

Preventive measures for dentin hypersensitivity

There is a well-known phrase: Prevention is better than cure. The search for advances toward better management of DH is ongoing, and better modes of treatment are becoming available. However, to date, the gold standard for DH that provides long-lasting and sustained improvement of the hypersensitivity condition has yet to be discovered. Among the many predisposing factors and etiologies for DH, incorrect tooth brushing technique is cited as the most common cause of DH.[6,24] Thus, dental education is essential to prevent DH and provide better oral health and overall well-being of the patient. Because DH may result from inappropriate/incorrect oral hygiene practices, educating the public on correct dental hygiene techniques may help prevent or improve DH. In addition, recommending healthier dietary habits to reduce or avoid acidic foods and beverages should be emphasized. Furthermore, destructive parafunctional habits, such as tooth grinding, which may result in erosive tooth structure loss, should be discouraged.

Diagnosis of dentin hypersensitivity

The diagnosis of DH is very subjective and difficult to establish despite several DH guidelines already published and available online.[4,24,25] The difficulty in diagnosing DH may partially be due to the numerous dental conditions that can present with symptoms similar to those of DH.[24] For this reason, DH is considered a diagnosis of exclusion—only when the signs and symptoms fail to meet the criteria of any other oral conditions or diseases can the diagnosis of DH be made. The sharp, sudden pain experienced upon consuming hot, cold, and sweet foods and beverages is a common complaint for both DH and reversible pulpitis. To a lesser extent, the symptoms of DH may also mimic those associated with tooth crack syndrome involving enamel or dentin, dental caries, post-bleaching/whitening dental hypersensitivity, and periodontal disease and its treatment.[26] As some patients may confuse the pain of DH with other types of pain, making an accurate diagnosis of DH requires a significant amount of time to take a proper and thorough history regarding the chief complaints and to conduct a complete intraoral clinical examination. Certain tests might also be required to exclude the other aforementioned oral conditions that mimic the symptoms of DH. Thus, a complete history and clinical examination are vitally important to obtain a definitive diagnosis of DH.

Since several common oral conditions can cause dental pain, distinguishing these conditions from DH is essential before a diagnosis of DH can be made. Some of the techniques that are commonly used to exclude the diagnosis of DH include eliciting a pain response upon percussion of the affected teeth (which would indicate periodontal or pulpal involvement), pain upon biting a tongue depressor, and the use of transilluminating lights or dyes (which would indicate tooth fractures). The presence of recent defective restorations may also be the source of pain. However, DH usually occurs at the area of gingival recession that is associated with loss of cementum on the root surface.[27]

After these common dental conditions that cause pain have been excluded, the diagnosis of DH can be confirmed by means of exposing the dentinal tubules to stimuli that mimic those that trigger DH. Some of the diagnostic tools that are commonly used for this purpose include the use

of an air or cold water jet, thermal testing, electrical devices, a dental explorer, a periodontal probe, radiographs, caries diagnostic devices, percussion testing, assessment of occlusion, and the bite stress test.[6,28]

Acknowledging a patient's complaints is the first step toward making a successful diagnosis and managing DH. Despite the high prevalence of DH, dental practitioners do not routinely assess their patients for this condition and rely more on the patient to self-report it.[6] Surprisingly, some dental practitioners even have a lack of confidence in diagnosing DH,[4] which could explain the low prevalence of DH in certain regions.

Soares et al. assessed the quality of life of people diagnosed with DH and concluded that DH was associated with a significantly high oral health impact.[3] In particular, the oral health impact was highest in terms of psychosocial discomfort, followed by physical pain, psychological disability, physical disability, social disability, and functional limitations. Thus, managing DH not only resolves patients' complaints but, more importantly, improves the physical and psychosocial aspects of their lives.

Treatment of dentin hypersensitivity

Since DH is caused by exposed dentinal tubules, occluding these tubules is the foundation of any therapeutic agents developed for DH treatment. Several treatment modalities are available for DH. They are classified by their mechanism of action – physical and chemical occlusion of the dentinal tubules, nerve desensitization, and photobiomodulation.

Physical occlusion of dentinal tubules

Physical occlusion of dentinal tubules is achieved by incorporating particles or nanoparticles into the dentinal tubules. Sources of particles that are commonly used for the physical occlusion of dentinal tubules include hydroxyapatite and bioactive glass. Hydroxyapatite composes approximately 70% of dentin's structure; thus, its use as a bone substitute and for tooth remineralization has shown promising results due to its biocompatibility and because it is nontoxic and harmless to human biological tissues.[29,30] Hydroxyapatite has been incorporated in desensitizing toothpaste as an agent to promote the repair of exposed dentinal tubules. Research is ongoing to further improve the effectiveness of hydroxyapatite in the management of DH.[31–33] Unlike hydroxyapatite, bioactive glass is an exogenous material that releases calcium and phosphate ions upon contact with fluids, such as saliva and water during drinking. These ions subsequently crystalize into hydroxycarbonate apatite to occlude the dentinal tubules.[34–36] Similar to hydroxyapatite, bioactive glass shows promising results as a bone substitute and for dentin remineralization, particularly due to its improved durability.[36–39]

Chemical occlusion of dentinal tubules

It has been widely reported that exposed dentinal tubules can also be treated with chemical agents, such as fluoride. Fluoride has long been known to play a vital role in preventing dental caries. Fluoride ions bind to calcium and phosphate by replacing the hydroxyl ion in the hydroxyapatite crystal structure, thereby forming a stronger enamel structure. In the management of DH, the use of desensitizing fluoride toothpaste has been recommended as the first-line noninvasive treatment.[4] Fluoride relieves DH by forming calcium-phosphate precipitates, calcium fluoride, and fluorapatite within the dentinal tubules, which prevents the intratubular fluid movement that would otherwise be triggered by the external stimuli that cause DH.[40–42] Other agents that regenerate hydroxyapatite similar to fluoride include oxalate, which is also commonly used in toothpaste and mouthwash for indirect occlusion of dentinal tubules.[43–45] Although chemical occlusion of dentinal tubules using fluoride, nitrate, and oxalate is common, its effects are not immediate and less efficient in withstanding acid challenges and mechanical stress.[32,46,47]

Nerve desensitization

Considering the proposed theory of nerve fiber involvement, nerve desensitization by means of applying potassium salts has become another treatment option for DH. Different formulations of potassium salts have been widely used in the management of DH. The use of potassium salts in DH was first introduced by Hodosh, whose clinical trial demonstrated that the use of 1–15% potassium nitrate (KNO3) was able to mitigate DH symptoms.[48] The mechanism of action of potassium salts in reducing DH symptoms in vitro and in human subjects has been discussed in depth elsewhere.[49,50] Briefly, increasing the concentration of potassium ions in the extracellular fluid around the peripheral dental afferent neurons depolarizes the nerve terminals and subsequently blocks conduction of an action potential.[50] Consequently, less pain is perceived in the brain, which improves the symptoms of DH. A systematic review and follow-up analysis on the effectiveness of different treatments for DH showed that the use of potassium nitrate desensitizing agents resulted in a significant long-term reduction in DH symptoms.[51]

Photobiomodulation

The concept of photobiomodulation for the treatment of DH was introduced by Matsumoto in the 1980s, who originally used neodymium-doped yttrium aluminum garnet (Nd:YAG) laser to treat cervical hypersensitivity.[52] This method is currently the most advanced technique for the treatment of DH. Nd:YAG is delivered by directing lasers of different wavelengths (ranging from ~600–800 nm) and laser powers (35–100 mW)[53] onto the exposed dentin.

In general, there are two types of photobiomodulation treatment depending on the laser power used for the treatment: low-level and high-level laser power. Low-power lasers such as gallium-aluminum-arsenide laser (GaAlAs) offer pain control in DH by increasing the production of mitochondrial ATP and increasing the threshold of the free nerve endings.[54] High-power lasers, such as carbon dioxide (CO_2), Nd:YAG, erbium-doped yttrium aluminum garnet (Er:YAG), and erbium, chromium-doped yttrium scandium gallium garnet (Er,Cr:YSGG), obliterate the dentinal tubules by inducing the formation of secondary and tertiary dentin produced by odontoblasts.[53,55] Studies have shown that when applied correctly, photobiomodulation with both low- and high-power lasers provides therapeutic relief for the treatment of DH without any detrimental effects to the pulp and dentin.[53,56]

Other treatment options

Depending on the clinical evaluation and assessment, DH can be managed with restoration when there is loss of tooth structure, such as due to abrasion. An appropriate choice of restorative materials should be used, or a root coverage procedure for DH caused by gingival recession can be performed. A systematic review of the effectiveness of surgical root coverage found reduced cervical DH following surgery[57]; however, more clinical studies are still required before surgical intervention is recommended for the management of cervical DH. In this regard, more recent clinical studies have demonstrated promising outcomes using a gingival flap combined with connective tissue grafts to augment and promote root coverage.[58–60]

Management of dentin hypersensitivity: How can successful outcomes be achieved?

Failure to treat its symptoms significantly affects the quality of life of patients suffering from DH. As prolonged hypersensitivity is manifested as pain, it may disturb an individual's daily activities, eating, drinking, and socializing. The resulting disruption of the patient's normal lifestyle may lead to the decision to remove the tooth causing the problem. Tooth loss may lead to even more functional impairment, as well as esthetic problems, depending on the location of the tooth. Depending on the type of materials used, tooth replacement can be a financial burden; thus, the vicious cycle of financial and psychosocial burdens continues. Identifying the factors that may lead to DH management failure may save a tooth from being removed and liberate the patient from a poor quality of life. Failure of DH management can be attributed to several factors, including dental practitioner factors,[4,24,61] patient factors,[62,63] and the methods and materials used.[51,64,65]

Dental practitioner

Making the correct diagnosis

Failure to identify the causative factor may lead to unsuccessful treatment of DH. Some dental practitioners overlook DH and manage their patients' complaints with invasive procedures. Mismanagement and misdiagnosis of DH cases may lead to irreversible loss of tooth structure and pulp vitality, or even tooth loss,[66] which could be prevented if an accurate diagnosis is made and the correct management performed. Dental practitioners should take a thorough history and perform a complete intraoral examination. This step is essential to establish the correct diagnosis, which must be achieved before treatment is initiated in patients.

Identifying factors that contribute to dentin hypersensitivity

It is now known that oral hygiene practices and dietary habits are significant etiologic factors that predispose individuals to DH. In addition to these well-accepted and well-known scientifically proven causes, an emerging association of DH with medication use has been reported. A recently published case report suggested an association between DH and steroid use. In this case report, a 47-year-old woman complained of symptoms similar to DH, i.e., generalized pain from all teeth, which was triggered by hot, cold and sweet foods, and beverages. The patient was on steroid treatment for her medical condition, and tapering and discontinuing the steroids improved her DH-like pain symptoms,[67] thus indicating that the DH was related to the steroids. Prior to this case report, a survey of 220 subjects was conducted to study the association between steroid use and DH. Surprisingly, almost 20% of the subjects reported DH-like symptoms similar to those in the aforementioned case report; the pain symptoms subsided following tapering and discontinuing of the steroids.[68]

In another study conducted by Farag and Awooda, 40 asthmatic patients who presented to an emergency department and were diagnosed with an acute asthmatic attack were monitored for DH and dental erosions.[69] The authors found significantly more DH in the asthmatic patients compared to the non-asthmatic patients. Studies have shown that the use of inhalers in asthmatic patients decreases salivary flow and salivary composition, which in turn reduces the buffering effect of saliva that protects tooth structures from erosion.[70–72] In this regard, the medications used to treat asthma, which include inhaled Beta2 agonists and inhaled steroids, may play a role in these salivary changes. For example, Beta2 agonists have been shown to impair saliva production[73] and lower esophageal sphincter function, which leads to gastroesophageal reflux.[74,75] Several studies, including a recent meta-analysis of clinical studies from 2012 to

2020, have suggested that gastroesophageal reflux is a risk factor for dental erosions.[76] The low pH of most inhalers also contributes to dental erosions and DH in asthmatic patients.[73] There is still limited literature about the association between DH and medical conditions and the use of certain medications. More studies are required to confirm this association and the possible mechanism that could link DH to medication use and/or other diseases.

Appropriate management

Similar to the management of other diseases, managing DH requires a holistic approach. Patients need to be motivated to change the habits that contribute to DH, and dental practitioners should consult with patients and develop appropriate and effective treatment plans. To ensure treatment success, both patients and dental practitioners must work together to eliminate the causative factors of DH and improve the discomfort and pain associated with this condition. Lack of commitment from either party—patient or dental practitioner—will lead to further tooth structure damage, for which some dental practitioners resort to a root canal.[77] Dental practitioners should keep themselves aware and updated about current advances in DH treatment. The choice of treatment options should consider the patient's conditions. Patients need to be evaluated with respect to their desire to improve their health and their motivation to achieve their treatment goals in order to ensure successful treatment. The initial management of DH often begins with patient education about the etiology and contributing factors, followed by in-office treatment in which chemical agents are professionally applied. Continuation with in-home treatment, which is cheaper and more convenient for the patient, is highly recommended to maintain the reduction of DH symptoms. However, when there is no improvement in symptoms, more advanced treatments, such as tooth restoration, root canal therapy, or even surgery, may be required. Dental practitioners need to assess and evaluate the most effective treatment methods and whether they should be provided by themselves or in combination with other treatments. Studies have shown that all types of treatment (physical occlusion, chemical occlusion, nerve desensitization, and photobiomodulation) significantly improved pain symptoms in DH patients compared to placebo group patients. However, there is no consensus in the literature regarding the effectiveness of one method compared to the others;[64,65,78–81] this divergence may be attributed to confounding factors, such as the type of study (in vivo or in vitro), study population, and study methods.

Patient habits and motivation

There are several risk factors for DH, including loss of tooth structure by erosion, attrition, abrasion or abfraction, bruxism, gingival recession, occlusal trauma, and abnormal tooth position.[82] These risk factors, in turn, are closely linked to the patient's dietary habits, such as frequent consumption of fruit juice, soft drinks, and alcohol, and tooth brushing habits, which include method of tooth brushing, type of toothbrush and toothpaste, and frequency of tooth brushing.[2]

In this regard, compliance and adherence to instructions are of paramount importance to ensure the success of DH treatment. Compliance and adherence are two different terms that are sometimes confused as having the same meaning. Compliance is defined as the consistency and accuracy with which a patient follows any regimen prescribed by a dentist, physician, or other healthcare professional.[83] Compliance does not reflect a stable state of the patient abiding by the healthcare provider's instructions. Therefore, compliance is complemented by adherence, which is defined as the extent to which the patient continues with the agreed-upon mode of treatment under limited supervision when faced with conflicting demands.[84] Thus, patients play a principal role in the treatment and management of their sensitive teeth, so they need to take control of their own efforts to modify unhealthy behaviors in order to reduce or eliminate DH symptoms. In this regard, dental professionals play a vital role in educating their patients and emphasizing the importance of compliance and adherence in changing behaviors and achieving a successful treatment outcome.

A patient's lack of motivation to comply and adhere to instructions provided by clinicians during therapy has been recognized as one of the main problems that prevent successful therapy. In their book chapter "Advances in the management of the patient with dentine hypersensitivity: Motivation and prevention", Gillam and Ramseier explain the challenges of modifying health behaviors in the management of dental conditions and outline their recommendations for managing DH using a motivational interviewing technique.[62] Motivational interviewing is defined as "a client (patient)-centered, directive method for enhancing intrinsic motivation to change by exploring and resolving ambivalence".[85] This technique helps patients recognize the importance of changing the detrimental habits that contribute to their DH and take ownership and responsibility of their problems through improvement of oral hygiene and dietary habits, i.e., by reducing acidic food and beverage consumption.[62]

While it may be unrealistic to expect patients to understand the professional advice on DH management, this can be accomplished by a few visits to a dental clinic. Although frequent dental visits may be inconvenient for patients, establishing good rapport during the first visit may help develop an effective dental practitioner-patient relationship and improve the patient's willingness to attend dental appointments.

Method chosen for the management of dentin hypersensitivity

A meta-analysis of the use of three DH management methods – physical occlusion, chemical occlusion, and nerve desensitization – for in-office and at-home treatment showed that only chemical occlusion and nerve desensitization were effective for at-home treatment. For in-office treatment, all three methods were very effective in managing DH compared to a placebo group.[80] A systematic review and network meta-analysis of in-office treatments for DH concluded that all methods of treatment were effective in reducing DH symptoms. The author reported no significant difference in the efficacy of the methods used to manage DH.[78]

Materials used for the management of dentin hypersensitivity

Regardless of the type of materials used to improve DH symptoms—by occlusion of dentinal tubules, nerve desensitization, or photobiomodulation—several factors need to be considered to ensure treatment success. These factors include, but are not limited to, physical properties, potential for microleakage, and durability of the materials. These materials should be able to withstand acidic environments (diet) and mechanical removal (tooth brushing) as well as avoid salivary clearance (dissolution in saliva).

A meta-analysis of the use of toothpaste as a desensitizing agent for DH found that incorporating potassium, stannous fluoride, potassium and strontium, potassium and stannous fluoride, calcium sodium phosphosilicate, arginine, and nanohydroxyapatite is effective in reducing sensitivity and improving symptoms compared to patients who used toothpaste without active ingredients.[86] However, the results may be biased in terms of the effectiveness of these active ingredients, as the studies had variable degrees of bias; some of them were funded by the manufacturers of the toothpastes studied.

Managing DH with photobiomodulation has gained considerable attention and is becoming more popular among dental practitioners. Many studies comparing lasers with other DH treatments support the use of lasers as being as effective as other techniques, including nerve desensitization and chemical and physical occlusion.[56,64,87,88] Several other studies have reported that combining laser therapy with any other method provides better outcomes for successful DH treatment.[76,79,89]

Suri et al. compared physical occlusion using 5% sodium fluoride with 980 nm diode laser treatment at one-week, one-month, and two-month intervals.[79] They determined that both methods improved DH symptoms, with no significant difference between methods. However, the combined use of 5% sodium fluoride and laser improved DH symptoms more than sodium fluoride or laser alone.[79]

Praveen et al. studied the application of glutaraldehyde topical desensitizer and compared it with low-level GaAlAs laser.[64] They concluded that both methods reduced DH immediately and after one week, but only GaAlAs laser improved symptoms after both one week and three months.[64] Interestingly, a clinical study conducted by Sgreccia et al. comparing potassium oxalate with low-level GaAlA laser treatment concluded that occluding dentinal tubules with potassium oxalate provided relief of DH symptoms immediately after the first application and after three weeks (with four applications), while laser was only effective in reducing cervical DH after three weeks.[90] These conflicting results may be due to the shorter duration of study by Sgreccia et al. compared to the other studies.

Lopes et al. assessed different DH treatment protocols that included low-level laser (with different doses), high-level laser, and desensitizing agents.[91] They studied 32 patients with 117 lesions who underwent one of the following DH protocols: low-power laser alone, high-power laser alone, or low-power laser combined with a desensitizing agent (Gluma desensitizer). The level of sensitivity was measured using a visual analog scale (VAS) for pain with a triple syringe and an exploration probe. The assessments were performed at intervals of five minutes, 12 months, and 18 months. At the end of their study, they found that all treatment protocols significantly reduced DH but that there was no significant difference in VAS scores between the different protocols used. Thus, they concluded that low-power laser, high-power laser, and low-power laser combined with a desensitizing agent were equally effective in reducing DH symptoms.

The photobiomodulation method used in the treatment of DH does not cause damage to the pulp tissue despite a slight rise in intrapulpal temperature, as reported in the systemic review of photobiomodulation effectiveness on DH by Machado et al.[53] Photobiomodulation has further been proven to improve the oral health-related quality of life of DH patients.[92]

Although a large body of evidence supports the use of photobiomodulation as superior to other DH treatments, given alone or in combination with other methods, many confounding factors affect its clinical success. A recent systematic review and meta-analysis of photobiomodulation parameters determined that the clinical outcome depends on variations in anatomy, site location, clinical condition, and subject individuality.[54] To achieve a better prognosis and successful treatment outcome, it is essential that these factors, combined with sound knowledge and understanding of laser parameters, tissue volume, and target depth to deliver an adequate dose, are considered during treatment planning.[54]

Photobiomodulation is known to be a safe nonsurgical therapy for DH that does not cause significant tissue hyperthermia. A recent randomized clinical trial reported a slight rise in intrapulpal temperature (<5°C) following

two diode laser protocols—1 W diode laser for 10 seconds or 0.5 W diode laser for 60 seconds.[81] Despite only a small rise in temperature, dental practitioners should be cautious when performing photobiomodulation for DH as any damage to pulp tissue can compromise tooth vitality and increase pain instead of relieve it.

Conclusions

DH is highly prevalent, especially in females. DH significantly affects the quality of life of many patients, which can impact their psychological and social well-being. Management of DH with a sound treatment plan is crucial to ensure successful treatment outcomes, which will improve the patient's oral health condition and quality of life. This review provides an update on the mechanisms of DH and its management and emphasizes the factors that determine the success of DH treatment. Pharmacological management of DH using desensitizing agents will only be successful if there is a concerted effort from both the dental practitioner and the patient.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

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Review

Green dentistry: Organic toothpaste formulations. A literature review

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Abstract

Dentistry as a profession should take into account the goals of sustainable development in daily practice and encourage the transition to a green economy. Consumers are becoming more conscious about the impact of self-care products, including toothpastes, on the environment. Organic toothpastes are considered very safe. A broad literature review was conducted to: (i) identify the ingredients in available organic toothpastes; (ii) classify them into active and inactive; and (iii) evaluate each ingredient's purpose and the adverse events that may be associated with its use. A comprehensive list of available organic toothpastes and their ingredients was compiled based on the products from the largest Italian organic supermarket chain (NaturaSi®) that is representative of the European market. Then, PubMed, Scopus and Google Scholar databases were searched to identify, classify and evaluate each ingredient. The final sample consisted of 46 organic toothpastes that included 156 ingredients; 139 (89.1%) and 17 (10.9%) were classified as active and inactive, respectively. Overall, 32 (20.5%) ingredients were associated with known adverse events. The results of this study indicate that organic toothpastes are highly biocompatible with oral cavity tissues. Careful product selection may help consumers avoid potential adverse effects that can be caused by ingredients such as polymers (e.g., polyethylene glycol) and carbomers, detergent agents (e.g., sodium lauryl sulfate), and triclosan. The lack of clinical studies should encourage the development of sufficient evidence to provide consumers with recommendations for daily use, based on both efficacy and biocompatibility.

Keywords: natural, toxicology, ingredients, organic toothpaste, plant/herbal extracts

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Introduction

In August 2017, the FDI World Dental Federation published a document titled "Sustainability in Dentistry"[1] that was based on the United Nations "Transforming our world: The 2030 Agenda for Sustainable Development".[2] It is recommended that dentistry as a profession takes into account the goals of sustainable development in daily practice and encourages the transition to a green economy.[3] Oral health professionals are responsible for reducing their impact on natural resources while promoting optimal oral health for all people and ensuring patient safety.[4] As a result, toothpaste formulations have dramatically improved over the last decade by incorporating safer active ingredients, higher fluoride bioavailability and better stain removal with fewer abrasives.[5] These products are supposed to simultaneously improve many oral diseases and conditions, such as caries, tooth discoloration, hypersensitivity, halitosis, and gingivitis, while also satisfying the expectations of more conscious and demanding consumers.[5]

The development of toothpastes, however, is far from complete. Over the last 20 years, the organic personal care market – of which the oral care subset represents a huge driving force – has grown exponentially and will presumably be nearly double by 2022.[6]

The requirements to be considered an "organic" product include: (i) the use of substances of natural origin characterized by ecological and skin compatibility and good aquatic toxicity performance; (ii) no genetically modified organisms in either the finished product or its individual ingredients; (iii) the use of natural fragrances (e.g., essential oils); (iv) the use of biodegradable detergents and surfactants; (v) no ionizing radiation; and (vi) the use of natural substances coming from controlled organic farming certified by a recognized accredited independent body.[7]

An attempt has been made to differentiate between active and inactive ingredients in widely marketed non-organic toothpaste formulations.[8] Active ingredients are expected to contribute to improved oral health, while inactive ingredients are added merely to enhance the appeal of the product. Unfortunately, a substantial proportion of inactive ingredients has been associated with adverse events (AEs), including enamel demineralization.[8] A previous review detailing the composition of non-organic toothpaste formulations found that 75.6% of the ingredients were associated with possible AEs.[8] In addition, nearly 30% of the ingredients were inactive.[8] Notably, most individuals were not aware of the risks associated with particular ingredients.[9]

Until now, no data about the content of active and inactive ingredients and the risks associated with organic toothpaste formulations is available in Europe.

Thus, this study aimed to: (i) identify the ingredients in available organic toothpastes; (ii) classify the ingredients into active and inactive; and (iii) evaluate each ingredient's purpose and the known risks associated with its long-term use.

Material and methods

Selection criteria for the organic toothpaste database

The products included in this study were analyzed based on the database from the largest Italian organic supermarket chain, NaturaSì®, which has more than 240 shops in the country. They were considered representative of the European market due to the international distribution of most of the industries in this field.

We accessed the online database of their dental products (https://www.naturasi.it/prodotti/cura-della-persona/igiene-orale) and extracted a list of available toothpastes. As this chain is restricted to organic products, all of the toothpastes surveyed had some European organic certifications. The first coder (MM) compiled the list of ingredients, and the second coder (AN) created a coding sheet to register all the ingredients of each toothpaste.

Identification of active and inactive ingredients

The authors developed an Excel (Microsoft Corp., Redmond, USA) database that included each ingredient for every selected formulation. Subsequently, each ingredient was classified as active or inactive, according to literature data obtained from PubMed, Scopus and Google Scholar databases from March 2021 to July 2021. In general, active ingredients are considered those typically present in toothpastes, such as emulsifying, buffering, preserving, and wetting agents, as well as thickeners, abrasives, solvents, absorbents, and antibacterial agents. Inactive ingredients include substances such as sweeteners, flavorings, colorants, and fragrance additives.

Ingredient toxicity

All AEs associated with each ingredient were collected by searching PubMed, Scopus and Google Scholar databases. Adverse events included the following: burning sensation/irritation/swelling/sensitivity of the cheek, tongue, lips, gum, palate, or papillae; tooth sensitivity; peeling/exfoliation/roughness of the cheek, tongue, lips, or gum; presence of aphthous ulcer/wounds; itching/tingling/taste changes of the cheek, tongue or lips; gastro-

intestinal signs and symptoms; and eye irritation. Since AEs may be individual in nature, the scope of the analyzed literature included not only randomized controlled trials and case-control studies on human and animal subjects but also case reports.

Database availability

The supplementary research database associated with this article (Excel database including each ingredient for every selected toothpaste formulation) can be obtained from the corresponding author on reasonable request.

Results

Organic toothpaste database

A total of 46 all-natural organic toothpastes sold by NaturaSì® were identified. They were classified according to different countries' certifications; 44 of the 46 toothpastes (95.7%) were marketed in more than 6 European countries. The toothpastes certified as all-natural organic products were NATRUE label, Biorganic DE, ICEA Eco Bio Cosmesi, BDIH, AIAB Bio Eco Cosmesi, Cosmebio, Ecocert, and Demeter. The Italian companies included Ecor, Natyr, Bioearth, Ecosì-Pierpaoli s.r.l., Victor Philippe, Lycon Cosmetics, and Argital. The German companies included Sante Naturkosmetik, Lavera Naturkosmetik, Logona Naturkosmetik, and Neobio. There was one product from each of the following countries: Switzerland (Weleda), England (Dr. Hauschka), the Netherlands (Ecodenta), and France (Cattier).

Identification of overall ingredients

A total of 156 unique ingredients were coded: 139 (89.1%) and 17 (10.9%) ingredients were classified as active and inactive, respectively. Overall, 32 (20.5%) ingredients were associated with known AEs; 23 were in the active group and 9 were in the inactive group.

The calculated mean number of ingredients per toothpaste was 16 ±3.85. The mean number of ingredients originating from plant extracts was 6.4 ±3.08.

Only 8 toothpastes (17.4%) were fluoridated; 6 (13.0%) contained sodium fluoride, and 2 (4.3%) had monofluorophosphate.

A total of 21 (45.7%) formulations contained xylitol, which was always among the first 10 ingredients.

Identification of active ingredients

One hundred thirty-nine of the 156 ingredients were classified as active. They were subdivided into emulsifiers, emollients, buffering agents, wetting agents, preserving agents, thickening agents, abrasives, whitening and plaque removal agents, solvents, absorbents, antibacterials, and active plant/herbal extracts. Among the 139 active ingredients, 23 (16.5%) were reported to have had possible AEs and 82 (59.0%) were organic plant/herbal extract ingredients. According to the literature search for each individual ingredient, none appeared to be related to any AEs.

Table 1 presents the classification of active ingredients according to their purpose and known AEs. Adverse events are listed in the table, when appropriate.

Table 1. Characteristics of the selected studies

Purpose and known adverse events of active toothpaste ingredients			
Type of ingredient	Ingredient	Purpose	Known adverse events
Active ingredient	sodium fluoride	anti-caries activity, whitening effects, halitosis control[1]	excess ingestion of sodium fluoride was linked with dehydration and with the possibility of dental and skeletal fluorosis[2–4]
	sodium monofluorophosphate	not found	not found
Emulsifiers	sodium lauryl sulfate	thickening and foaming agent[5]	skin, eyes, oral mucosa, and gastrointestinal irritant[6–8]
	sodium carboxymethyl cellulose	emulsion stabilizing agent[9]	no known contamination risks[9]
	poloxamer 407	emulsifying agent[10,11]	eye and renal irritant[12,13]; in animal studies and with parenteral administration, it was linked to hyperlipidemia and engorgement of Kupffer cells[14]
	tetrasodium pyrophosphate	anti-biofilm action by reducing saliva calcium and magnesium[15,16]	nose, skin, eye, throat, and respiratory tract irritant[17]
	sodium hexametaphosphate	extrinsic stain removal[18]	skin, eye, respiratory tract irritant; can cause gastrointestinal symptoms and lethargy, when ingested[19]
	sodium cocoyl glutamate	surfactant	not found

Purpose and known adverse events of active toothpaste ingredients			
Type of ingredient	Ingredient	Purpose	Known adverse events
Emulsifiers	disodium cocoyl glutamate	surfactant	not found
	disodium cocoamphodiacetate	surfactant	not found
	algin	thickener	not found
	sodium chloride	moisturizer	not found
	pistacia lentiscus gum	antibacterial activity[20]	not found
	Cyamopsis tetragonoloba (guar) gum	natural thickener	not found
	sodium lauroyl glutamate	surfactant	not found
	lauryl glucoside	foaming agent	allergic dermatitis reported in case studies[21]
	sodium lauroyl sarcosinate	pH buffering agent	non-irritating and non-sensitizing to animal and human skin; can increase the penetration of other ingredients through the skin; low oral toxicity in rats, not mutagenic; no data on carcinogenicity[22]
	betaine	moisturizer and foam stabilizer[23]	not found
	hectorite (natural clay)	excellent absorption capacity[23,24]	not found
	magnesium aluminum silicate	excellent absorption capacity	not found
	sucrose laurate	solubilization properties[25]	not found
Emollients	*Triticum vulgare* (wheat) germ oil	emollient and antioxidant agent; often mixed with other oils, and used as a base for many creams for mature skin, owing to the high content of tocopherols (vitamin E) which also makes it an excellent antioxidant[23]	not found
	esculin	glucoside extracted from *Aesculus hippocastanum, Aesculus californica* and *Daphne mezereum*[23]	not found
	Helianthus annuus oil (sunflower)	rich in linoleic acid, vitamin E, vitamin A, a powerful antioxidant, helps to maintain the right cholesterol level and protects against cardiovascular diseases by keeping the blood flowing[26,27]	not found
	potassium nitrate	desensitizing agent [28]	not found
Buffering agents	disodium pyrophosphate	remineralizing agent	not found
	sodium hydroxide	buffering agent	unclear toxicity; at high concentrations sodium hydroxide has been shown to affect the viability of esophageal cells[29]
	calcium citrate	remineralizing agent	not found
	sodium silicate	remineralizing agent	extremely toxic in purity to contact[30] [Unclear. Could you rephrase?]
Preserving agents	sodium benzoate	preserving agent	excess consumption could decrease the functioning of the immune system and cause other irritations[31,32]
	potassium sorbate	preserving agent	dangerous in case of ingestion and inhalation; skin and eye irritant[33]
	sodium dehydroacetate	preserving agent	allergic dermatitis reported in case studies[34]
	ethylhexylglycerin	preserving, antibacterial, surfactant, skin-conditioning, emollient agent	a low-risk but relevant sensitizer in 'hypoallergenic' formulations[35]
	salicylic acid	preserving agent	salicylic acid toxicity (salicylism) can occur after topical use of 6% salicylic acid over as little as 40% of body surface area[36]
	sorbic acid	preserving agent	contact urticaria [after the use of?] synthetic cassia oil and sorbic acid limited to the face[37]
Thickening agents	cellulose gum	thickening agent[9]	skin and eye irritant[38]
	xanthan gum	thickening and stabilizing agent[39]	xanthan gum in purity [pure xanthan gum?] could cause bloating, cold and flu-like symptoms[40,41]
	carrageenan	thickening agent[9]	gastrointestinal issues[42–44]

Purpose and known adverse events of active toothpaste ingredients			
Type of ingredient	Ingredient	Purpose	Known adverse events
Abrasives	calcium carbonate	active in extrinsic stain and plaque removal[45]	eye and respiratory tract irritant [46]
	silica	abrasive agent[47]	not found
	diatomaceous earth (solum diatomeae)	abrasive agent	not found
	illite	abrasive agent	not found
Whitening/plaque removal agents	citric acid	active in dental plaque removal[9]	the developmental toxicity associated with chronic consumption of citric acid is not known[48]; gastrointestinal symptoms are reported to be associated with citric acid consumption[9]
	hydrated silica	active in extrinsic stain and plaque removal[49]	use of silica nanoparticles in-vivo poses risks of bioaccumulation[50]
	mica	not found	not found
	sodium bicarbonate	active in extrinsic stain and plaque removal[51]	rare reactions such as dizziness, confusion, irritability, memory problems, muscle pain or aches, vomiting, or weakness are reported to be associated with its excess consumption[52]
	titanium dioxide	active in extrinsic stain removal[53]	mixed findings; recent work suggested that titanium dioxide in higher concentrations may be dangerous[53]; particularly, chronic health effects include possible harm to the upper respiratory tract and lungs[54]
	trisodium phosphate	not found	not found
	xylitol	active in caries prevention[55]	no carcinogenicity[55]
Wetting agents	glycerin	used to prevent toothpaste from drying out[56]	generally used with low toxicity, but at high concentrations, it could impair blood circulation[57]
	hydrogenated starch hydrolysate	used also as a sweetener (polyol (sugar alcohol))	not found
	lactose	used also as a sweetener	not found
	calcium lactate	used also as an enamel remineralizer	not found
Solvents	aqua	solvent	not found
	denatured alcohol	solvent	not found
	alcohol	solvent	not found
Absorbents	kaolin	cleaning and polishing agent	not found
	solum fullonum	absorbent	not found
	maltodextrin	used also as a sweetener	not found
	charcoal powder	buffering agent	not found
Antibacterials	*Copaifera officinalis* resin	antibacterial agent	not found
	honey (manuka honey IAA15+)	antibacterial agent	not found
	Anthemis nobilis extract (chamomile)	antibacterial, antifungal, insecticidal, hypotensive, anti- platelet aggregation, anti-inflammatory, hypoglycemic, and antioxidant agent[58]	the US Food And Drug Administration (FDA) classified the oil and extract of Roman chamomiles as safe[58]
	bisabolol	antibacterial and thickening agent	not found
	carvone	antibacterial agent	not found
Other ingredients	Aloe vera	antioxidant and antibacterial properties	not found
Vegetable-based ingredients	*Mentha arvensis* oil	analgesic counterirritant	not found
	Mentha arvensis flower/leaf/stem extract	analgesic counterirritant	sensitizer; case reports on allergic contact cheilitis caused by toothpaste menthol[59]
	Mentha viridis (spearmint) leaf oil	analgesic counterirritant	sensitizer and allergenic[59]
	Melissa officinalis flower/leaf/stem water	antimicrobial and antioxidant agent	case reports on contact dermatitis[60]
	Aloe barbadensis leaf juice	emollient and antimicrobial agent; used to treat aphthous ulcers and to reduce the incidence of alveolar osteitis after third molar extraction surgeries[61]; a mouthrinse containing *A. vera* was found to reduce gingival inflammation and gingival bleeding[62] and was more effective than Listerine® in reducing the count of aerobic, microaerophilic and anaerobic bacteria[63]	not found

Purpose and known adverse events of active toothpaste ingredients			
Type of ingredient	Ingredient	Purpose	Known adverse events
Vegetable-based ingredients	*Aloe barbadensis* gel	emollient, antimicrobial	not found
	Mentha piperita oil	analgesic, antiseptic and anti-inflammatory properties[64]	not found
	Myrtus communis leaf water	antibacterial activity on oral pathogens: *Streptococcus mutans, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Streptococcus pyogenes* and *Candida albicans*[65]	not found
	Echinacea purpurea extract	immunostimulant, increases interferon production[66]	not found
	Salvia officinalis (sage) leaf extract	antioxidant	not found
	Salvia officinalis oil	antioxidant	not found
	Salvia sclarea (clary) oil	antioxidant	not found
	Salvia triloba (sage) leaf oil	antioxidant	not found
	Mentha piperita (peppermint) leaf extract	analgesic counterirritant	not found
	Glycyrrhiza glabra root extract	anti-inflammatory activity	not found
	Camellia sinensis leaf water	antimicrobial	not found
	Camellia oleifera leaf extract	antimicrobial	not found
	Mentha spicata herb oil	analgesic counterirritant	not found
	Krameria triandra extract	astringent	not found
	Elettaria cardamomum seed oil	active on oral cavity disinfection and halitosis; regulates inflammatory and immune function[67]	not found
	Citrus limon peel oil	antibacterial agent	not found
	Achillea millefolium extract	hydrating agent	not found
	Echinacea angustifolia root extract	immunostimulant agent: increases interferon production	not found
	Echinacea pallida extract	immunostimulant agent: increases interferon production	not found
	Arnica montana flower extract	antimicrobial, anti-inflammatory, antibiotic and antifungal agent	not found
	Melaleuca alternifolia leaf oil	antimicrobial agent	not found
	Fragaria chiloensis fruit extract	astringent effect	not found
	Rubus idaeus fruit extract	anti-biofilm formation	not found
	Rosmarinus officinalis (rosemary) leaf extract	anti-biofilm formation	not found
	Rosmarinus officinalis oil	anti-biofilm formation	not found
	Citrus aurantium bergamia (bergamot) peel oil	fragrance agent	not found
	Citrus grandis (grapefruit) peel oil	antimicrobial activity: inhibits metabolism of and kill plaque bacteria	not found
	Citrus aurantium dulcis (orange) peel oil	fragrance agent	not found
	Thymus vulgaris oil	antimicrobial activity comparable to clorexidine or triclosan against *S. mutans*[68]	not found
	Eugenia caryophyllus (clove) leaf oil	antimicrobial agent	not found
	Chamomilla recutita flower extract	anti-inflammatory agent	not found
	Pimpinella anisum oil	antioxidant and antimicrobial agent[69]	not found
	Foeniculum vulgare fruit extract (fennel)	antibacterial agent active on *S. mutans*[70]	not found
	Hamamelis virginiana flower water	antioxidant and anti-inflammatory agent	not found
	Malva sylvestris (mallow) extract	active against *S. mutans* and *S. aureus*[71]	not found

Purpose and known adverse events of active toothpaste ingredients			
Type of ingredient	Ingredient	Purpose	Known adverse events
Vegetable-based ingredients	*Commiphora abyssinica* resin extract	antiseptic agent	not found
	Commiphora myrrha extract	antiseptic agent	not found
	Malva officinalis flower extract	active against *S. mutans*, *S. aureus*[71]	not found
	Calendula officinalis flower extract	active in preventing gingivitis, periodontal disease, stomatitis, and halitosis[72]	not found
	Illicium verum fruit/seed oil (star anise)	antibacterial and antifungal activity, especially active on *Staphylococcus aureus*[73]	not found
	Rosa canina fruit extract	antibacterial, antioxidant, astringent agent	not found
	Avena sativa extract	humectant agent	not found
	Chondrus crispus (carrageenan) extract	antimicrobial agent	not found
	Aesculus hippocastanum (horse chestnut) seed extract	matrix metalloproteinase inhibitor[74]	not found
	Carbon of beech and *Betulla*	whitening efficacy	not found
	Aesculus hippocastanum (horse chestnut) bark extract	matrix metalloproteinase inhibitor[74]	not found
	Arum maculatum root extract	antimicrobial activity against *S. aureus*[75]	not found
	Melia azadirachta leaf extract	anti-biofilm activity	not found
	peat moss extract	anti-biofilm activity	not found
	Prunus spinosa fruit juice	phenolic components and antioxidant activity[76]	not found
	Simmondsia chinensis (jojoba) seed oil	antimicrobial activity against *S. aureus*	not found
	Juglans regia (walnut)	anticariogenic[77] and anti-*Candida albicans* activity[78]	not found
	papain	anticariogenic,[79] anti-biofilm formation; effectively digests the main actinomyces fimbrial proteins, fimP and fimA[80]	not found
	bromelain	antimicrobial,[81] antiplaque and antigingivitis,[82] adjuvant in treatment of periodontitis[83]	not found
	Petroselinum sativum oil	antimicrobial agent active on Streptococcus mutans[84]	not found
	Berberis vulgaris extract	antimicrobial agent[85]; inhibits collagenase activity of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*[86]	not found
	Eucalyptus globulus oil	anti-inflammatory agent	not found
	Cinnamomum zeylanicum oil	active against *S. mutans*, *S. aureus* and *Candida albicans*	not found
	stevioside	natural sweetener[87]	not found
	Glycyrrhiza glabra (licorice) root extract	anti-inflammatory, antioxidant[88]	not found
	Leptospermum scoparium branch/leaf oil (Manuka oil)	strong antibacterial activity against periodontopathic and cariogenic bacteria (*Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum,* S. mutans, and *S. sobrinus*)[89]	not found
	Colocasia antiquorum extract	antioxidant, effective in the treatment of aphthous ulcers[90]	not found
Biological additives	maris sal (dead sea salt)	saliva flow stimulation	not found
	propolis cera	mucositis-effective[91]	not found
	Ammonium glycyrrhizate (licorice root)	anti-inflammatory, antioxidant[88]	not found
Antioxidants	xanthophyll	antioxidant	not found
	Styrax benzoin extract	antioxidant[92]	not found
Vitamins	cyanocobalamin (vitamin B12)	[93]	not found

Identification of inactive ingredients

Seventeen of the 156 ingredients were classified as inactive. They were subdivided into sweeteners, flavorings, natural colorants, and fragrances. Of these, 9 (52.9%) were reportedly associated with a known AE, while 8 (47.1%) were not.

Among the inactive ingredients, 14 (82.4%) were natural ingredients, including organic essential oils (n = 8), natural colorants (n = 3; natural red, Carbo vegetabilis and natural green (chlorophyll) – CI 75810), natural flavoring agents (n = 2), and one natural sweetener (*Stevia rebaudiana*).

The reported AEs were skin and eye irritations (coumarin, citronella, geraniol, linalool, cinnamaldehyde, and limonene) and gastrointestinal symptoms (sorbitol).

Table 2 presents the inactive ingredients with their purposes and known AEs. Adverse events are listed in the table, when appropriate.

Table 3 presents the classification of the ingredients and the percentage of AEs among the groups.

The research dataset is available from the corresponding author on reasonable request. All the ingredients are classified and listed in the table according to each of the examined formulations. Moreover, the International Nomenclature of Cosmetic Ingredients (INCI) for each of the examined formulations is reported.

Discussion

This study identified for the first time the ingredients of 46 all-natural organic toothpastes currently marketed in Europe and classified them into active and inactive compounds. The study also evaluated, through a broad literature review, the purpose of the ingredients, as well as their known toxicity risks. In total, 156 unique ingredients were analyzed: 89.1% (n = 139) and 10.9% (n = 17) were classified as active and inactive, respectively.

According to our study, only 20.5% of the ingredients found in the organic toothpastes were associated with AEs, reassuring the safety of these products for oral care.

Table 2. Description of inactive ingredients with possible adverse events

Purpose and known adverse events of inactive toothpaste ingredients			
Type of ingredient	Ingredient	Purpose	Known adverse event
Sweeteners	sodium saccharin	artificial sweetener[1]	mixed findings; considered safe for consumption,[1] but new research states that it contributes to enamel demineralization[2]
	Stevia rebaudiana (leaf) extract	bio-sweetener,[3] antioxidant activity[4]	not reported
	sorbitol	substitute for sugar used as a sweetening agent[1]	reported to cause abdominal discomfort[5]
Flavorings	flavor	functions as a flavoring agent[6]	has been shown to cause allergic reactions, such as inflammation of the mouth and lips and gingivitis[6]
	menthol	functions as a flavoring and scent for a toothpaste[7]	no known contamination risks[7]
	Commiphora myrrha resin extract	functions as a flavoring and scent for toothpaste and a natural anti-septic	no known contamination risks[8]
Natural colorants	CI 75810 (natural green (chlorophyll))	natural green pigment	no known contamination risks[9]
	CI 77268:1 (Carbo vegetabilis)	cosmetic colorant	not reported
	CI 75470 (natural red)	cosmetic colorant	not reported
Fragrances	limonene	flavor and fragrance additive,[10] derived from *Citrus aurantiifolia* oil	generally recognized as safe; case reports on irritant effect on eyes, nose, throat and skin; also, found to cause kidney damage in rats, but similar results were not found in humans[7]
	cinnamaldehyde	flavor and fragrance additive	strong evidence: human immune system toxicant or allergen; not suspected to be bio-accumulative[11]
	linalool	flavor and fragrance additive	skin, eye and respiratory tract irritant; not suspected to be bio-accumulative[11]
	eugenol	flavor and fragrance additive	classified as safe[12]
	citral	derived from *Citrusaurantiifolia* oil flavor and fragrance additive	no known contamination risks
	geraniol	flavor and fragrance additive	dangerous in case of ingestion and inhalation; marginally hazardous in case of skin contact (permeator), skin and eye irritant[11]
	citronella	flavor and fragrance additive	hazardous in case of ingestion; skin and eye irritant
	coumarin	flavor and fragrance additive	extremely hazardous in case of ingestion and inhalation; skin and eye irritant; severe overexposure can result in death; potential chronic carcinogenic effects (classified 2B, possible for humans)[13]

Table 3. Classification of ingredients

Ingredients		*n*	Safe	With AEs
Active	Fluorides	2	1 (50%)	1 (50%)
	Emulsifiers	19	12 (63.15%)	7 (36.85%)
	Emollients	4	4 (100%)	0
	Buffering agents	4	1 (25%)	3 (75%)
	Preserving agents	6	0	6 (100%)
	Thickening agents	3	0	3 (100%)
	Abrasives	4	3 (75%)	1 (25%)
	Whitening/plaque removal agents	7	2 (28.6%)	5 (72.4%)
	Wetting agents	4	3 (75%)	1 (25%)
	Solvents	3	3 (100%)	0
	Absorbents	4	4 (100%)	0
	Antibacterials	5	5 (100%)	0
	Active plant/herbal extracts	74	71 (96%)	3 (4%)
	Total	139	109 (78.4%)	30 (21.6%)
Inactive	Sweeteners	3	1 (33.3%)	2 (66.7%)
	Natural colorants	3	3 (100%)	0
	Flavorings	3	2 (66.7%)	1 (33.3%)
	Fragrances	8	2 (25%)	6 (75%)
	Total	17	8 (47%)	9 (53%)

AEs – adverse events.

In detail, the study determined that most of the potential toxicity of the products was in the inactive ingredients.

The findings of this study are relevant to the following issues:

1. Compared with the results of the Basch and Kernan study on the composition of non-organic toothpastes for children, the percentage of ingredients associated with possible AEs in this study was much lower (75.6% compared to 20.5%). The Basch and Kernan study identified 71.1% and 28.9% of all components as active and inactive ingredients, respectively, while they were 89.1% and 10.9%, respectively, in this study.[8]

2. Most of the possible AEs associated with non-organic products do not occur with organic toothpastes. In particular, only 2 of the toothpaste formulations evaluated in this study contained the 2 classes of ingredients most frequently associated with toxicity, namely, synthetic polymers (e.g., polyethylene glycol (PEG), carbomers and Triclosan) and detergent agents (e.g., sodium lauryl sulfate (SLS)). This indicates favorable biocompatibility for the other 44 products. Synthetic polymers and detergent agents were present in only 2 formulations by one manufacturer (Cattier). The literature reports that nonionic surfactants (e.g., PEG) act as penetration enhancers by decreasing surface tension and conditioning the stratum corneum, which may increase the diffusion of other molecules through the skin.[10] Moreover, there is evidence on the side effects of detergent agents (e.g., SLS) that can cause irritation and soft tissue peeling.[11–13] Sodium lauryl sulfate is reported to be the most toxic agent on mucosal cells; it causes epithelial desquamation,[14,15] as shown by studies on the cytotoxicity and genotoxicity of commercial toothpastes.[16–18]

This study did not identify any artificial colorings, such as FD&C Blue No. 1 Lake, D&C Red No. 30 Lake, D&C Red No. 28, and Red No. 40, which are associated with allergic reactions, neurotoxicity, carcinogenicity, and skin discoloration.[19–21] In addition, there were no foam or flavor boosters, such as cocamidopropyl betaine, in the organic toothpaste formulations. Although rare, these compounds can cause allergic reactions.[22,23]

3. Most potential AEs associated with organic products are due to inactive ingredients, such as fragrances that can cause skin and eye irritations. In addition, the authors need to highlight that the study searched for any potential toxicity, regardless of the dose. This results in the conclusion that the very low absolute dose in these toothpastes is unlikely to cause such AEs. Furthermore, detailed information on the concentration (%) of each ingredient is missing, which is challenging for researchers.

Twenty-seven (58.7%) formulations contained a sweetening agent, including 25 (92.6%) with sorbitol, one (3.7%) with sodium saccharine, and one (3.7%) with *Stevia rebaudiana*. *Stevia rebaudiana* is a sweetening agent that has no reported AEs, so it meets the safety standards of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Recent research suggests that sodium saccharine, although safe for consumption,[24] contributes to enamel demineralization.[25] Sorbitol has been shown to cause abdominal discomfort and diarrhea.[26] Conversely, a recent review by de Cock et al.[27] highlights the extensive research on the safety of erythritol, a polyol produced by the natural process of fermentation. Erythritol has sweetness and calorific reduction compared to sucrose, no cariogenic potential, a low glycemic index, a relatively high stability in acidic and alkaline environments, a high stability against heat, and suitability as a bulking agent in food manufacturing. Furthermore, there is a significant number of toxicology and safety studies that report a lack of AEs associated with small amounts of erythritol.[9,28,29] Analogous characteristics of safety and health effects are valid for xylitol. Considering this evidence, the authors propose the use of *Stevia rebaudiana*, erythritol or xylitol in toothpaste formulations instead of other components that have been linked to AEs.

Regarding fragrances, the authors suggest focusing on 100% naturally sourced, plant-based essential oils to avoid possible AEs. Moreover, they recommend that as small a quantity as possible should be used to avoid potential AEs.

Essential oils should have 3 major characteristics: safe to consume, pure food grade, and organic certification. Alternatively, naturally derived flowers, spices and fruit flavors are suitable options, especially for children's toothpaste.

In the past, the risk of bioaccumulation had been correlated with the amount of toothpaste inadvertently swallowed during brushing.[30] Consequently, the American Dental Association (ADA) recommendations stated that no amount of toothpaste should ever be swallowed intentionally.[31] Therefore, based on the results of a recent randomized placebo-controlled study that evaluated the effect of a vitamin B12-fortified toothpaste on vitamin status markers in vegans, manufacturers should be cautious when selecting toothpaste ingredients. This is because each toothpaste ingredient, when in contact with the oral cavity, can also enter the systemic blood circulation via the sublingual blood system route. The study demonstrated that, compared to the placebo, the vitamin B12-fortified toothpaste resulted in a significant increase in serum vitamin B12 concentration after 12 weeks. Although the authors specified that the mechanism of vitamin B12 absorption via the mucosal barrier is currently not known, the toothpaste-based strategy may be a promising approach for delivering vitamins.[32]

While there are few existing trials evaluating the clinical effectiveness of organic toothpastes, there appears to be a rising trend. It is necessary to obtain scientific evidence of abrasive action, removal of external discoloration, and remineralizing effects on enamel. Interestingly, only 8 (17.4%) of the toothpastes analyzed in this study were fluoridated: 6 (13%) with sodium fluoride and 2 (4.3%) with monofluorophosphate. The remineralizing effect of fluoride on International Caries Detection and Asessment System (ICDAS)-II codes 1 and 2 early carious lesions is well understood.[33] Considering this data, many questions and doubts arise about the remineralizing effects of organic toothpastes; answering them as soon as possible is fundamental to promote their use and provide both clinicians and patients with clinical recommendations based on evidence.

A recent systematic review highlighted the efficacy of *Camellia sinensis* extracts on gingivitis and periodontitis.[34] Thus, it is surprising that it was present in only 2 of the 46 toothpaste formulations analyzed in this study. A recent clinical study found that scaling and root planing with the aid of ozonated olive oil mouthwash were more effective on salivary matrix metalloproteinase-8 (MMP-8) reduction than scaling and root planing alone.[35] None of the examined formulations contained ozonated olive oil. The supplementary research database is available from the corresponding author on reasonable request. All of the listed single ingredients can be searched.

In the light of the above, the authors of this article emphasize the need for direct comparisons between manufacturers and researchers. It is only through constant dialogue and inclusive cooperation between the manufacturers and those conducting the research that products requested by patients can be obtained. In fact, patients are increasingly oriented toward ecological transition and sustainability, given the development of dentistry itself toward less invasive procedures that also consider the patient's quality of life.[36–38]

Limitations

The main limitation of this study is that it only included one supermarket chain in one country. However, the authors showed that most products are marketed in more than 6 European countries, which diminishes the impact of this limitation. Another limitation is that the toxicity evaluation was based on evidence emerging from our literature search, which did not assess the actual quantity (see above) or possible combinations; these could be considered worst-case scenarios. Third, the authors did not conduct a direct comparison between organic and non-organic toothpaste formulations. However, the difference between the analysis in this study and previous analyses is sufficient to minimize this bias. Finally, the efficacy was not assessed. Future research is warranted to further evaluate organic toothpastes and address these limitations.

Conclusions

Based on the results of this study, the organic toothpaste formulations showed adequate safety when each ingredient was analyzed. However, the clinical efficacy of the organic toothpastes still needs to be assessed to support their daily use for maintaining oral health and preventing diseases. This analysis provides important information for consumers who are concerned with ecological and environmental issues.

Ethics approval and consent to participate

Not applicaple.

Data availability

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

Consent for publication

Not applicable.

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Review

Role of implant loading time in the prevention of marginal bone loss after implant-supported restorations: A targeted review

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Abstract

The implant-supported restoration of missing teeth is a recognized method of treatment that ensures a functional, esthetic and durable effect, along with patient satisfaction. However, the preferable time of dental implant loading is under debate. Currently, 3 protocols are used: immediate loading; early loading; and conventional (late) loading. Immediate loading provides benefits such as short treatment time, the elimination of the second surgery required for later loading protocols, the protection of the gingival papilla, an immediate esthetic effect, and high patient satisfaction. This review aimed to summarize the evidence on the impact of loading time on marginal bone loss (MBL) around dental implants, which is considered a useful measure of implantological treatment effects. A literature search was conducted based on the PubMed/MEDLINE database. The search focused on studies providing the MBL values by protocol. Out of the 1,366 hits received in the initial search, 10 studies were included in the qualitative analysis. At 12 months, the MBL range was 0.17–1.86 mm in patients undergoing the immediate protocol, 0.14–1.22 mm in patients undergoing the early protocol, and 0.44–0.91 mm in patients undergoing the late protocol. The studies were heterogeneous, but no significant differences in the occurrence of MBL were reported between the immediately and early loaded implants as compared with the conventionally loaded ones. Further studies are needed to determine other factors that might be related to the type of protocol, important for optimal patient treatment.

Keywords: dental implants, marginal bone loss, loading protocol, loading time

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Introduction

Dental treatment involving implants is an effective and widely used method of restoring missing teeth, which may result from untreated caries, injuries, tumors, or congenital defects (hypodontia). The reconstruction of missing teeth with the application of implants is a recognized method of treatment. It ensures functional, esthetic and durable restoration, along with patient satisfaction. Over the years, efforts have been made to shorten the duration of implant treatment.

Currently, success in implant therapy is not only based on the implant survival rate and the condition of the tissue in direct implant vicinity, as previously suggested by Albrektsson et al.[1] and Buser et al.,[2] but also on the full esthetic effect and patient satisfaction. The peri-implant soft tissue, i.e., the pink esthetic score (PES), is assessed using 5 parameters: the mesial papilla; the distal papilla; the curvatures of the facial mucosa; the level of the facial mucosa; and root convexity/soft tissue color and texture. To evaluate the white esthetic score (WES) of the visible portion of the implant restoration, 5 parameters are taken into consideration: the tooth form; the outline/volume of the clinical crown; color; surface texture; and translucency. Each of the 5 parameters of PES and WES was graded with a 0-1-2 score, and consequently, the assessment resulted in the lowest score of 0 and the highest score of 10 for each of the 2 indices.[3] High PES and WES scores indicate good condition of soft tissues and esthetic prosthetic reconstruction. In implant-supported prosthetic reconstruction, we can distinguish 3 protocols: immediate loading; early loading; and conventional (late) loading.[4] All 3 protocols are used for different types of restorations – single, multiple and full-arch.

The extent of marginal bone loss (MBL) is the basic indicator of peri-implantitis. Marginal bone loss is measured from the neck of the implant to the first bone-to-implant contact and is determined during a radiological examination. Marginal bone loss has been considered a useful measure to evaluate the effects of treatment with implants. This review aimed to summarize the current evidence on the impact of loading time on MBL around dental implants.

Methods

A literature search was conducted in the PubMed/MEDLINE database with the use of advanced search options. Only publications in English, studies on humans with an observation period of at least 12 months as determined by authors, and studies published between January 2002 and June 2021 were considered. No restrictions in terms of geographical scope were imposed. Only full-text articles were included; abstracts and posters were excluded. The following search terms and their combinations were used: 'implant loading'; 'protocol'; and 'marginal bone loss'. The inclusion criteria are reported according to the PICOS criteria and are presented in Table 1. The search was run on February 17, 2022. The list of titles, abstracts and full texts was reviewed by one reviewer according to the defined inclusion and exclusion criteria to identify and select articles related to the topic of interest. The non-systematic approach was used with regard to the qualitative analysis of the identified publications. The extracted data included the loading protocol, the MBL values and the factors affecting the degree of MBL.

Table 1. Inclusion and exclusion criteria

PICOS model	Inclusion criteria	Exclusion criteria
Population	adult human population	animal models
Intervention	implantological treatment	–
Comparison	controlled or single-arm studies	–
Outcome	MBL evaluated during at least 1 year of follow-up	follow-up below 1 year
Study design	RCT, cohort study, case–control study, prospective and retrospective studies	review, meta-analysis, letter to the editor, editorial, opinion

MBL – marginal bone loss; RCT – randomized clinical trial.

Results

After searching PubMed with the use of the keywords related to implantological treatment and its association with MBL, we received 1,366 hits, of which 10 were included in the qualitative analysis. The flowchart of the study is depicted in Fig. 1.

Overall, 10 studies met the inclusion criteria and reported values for MBL, along with definitions of loading protocols; however, they presented data for different follow-up periods and different patient populations. Overall, at 12 months, the MBL values ranged from 0.17 mm

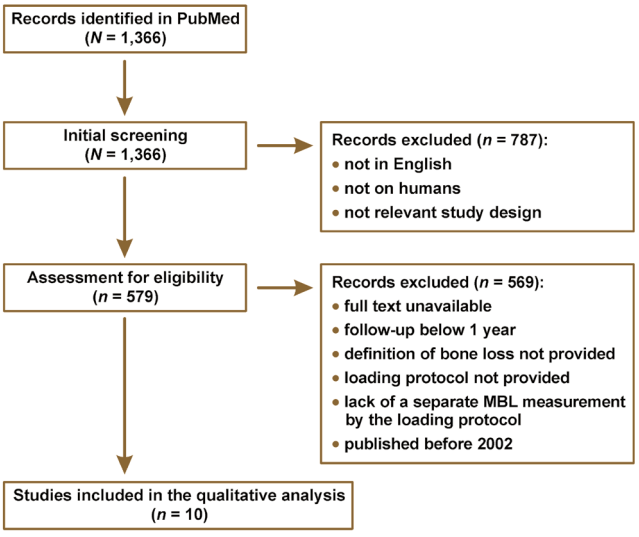

Fig. 1. Flow chart of the study selection process for the systematic review

to 1.86 mm in patients undergoing the immediate protocol, from 0.14 mm to 1.22 mm in patients undergoing the early protocol, and from 0.44 mm to 0.91 mm in patients undergoing the late protocol. The summary of the studies is presented in Table 2.

Discussion

Our review showed that there is still insufficient evidence on the impact of the implant loading protocols on maintaining healthy bone tissue as measured with MBL. Most identified studies concluded that differences in MBL between patients receiving implants according to different loading protocols were not significant. However, a great heterogeneity was observed among studies. They differed in terms of follow-up periods, characteristics of the populations studied, sample sizes, and other factors related to treatment.

Immediate loading

In the literature there is no agreement on the time at which a prosthetic construction is attached to the implant. Ostman defines immediate loading, also called direct loading, as attaching a prosthetic construction to the implant up to 24 h after implantation.[15] Galli et al. considered 48 h as the maximum time of the restoration placement on the implant.[8] According to Esposito et al., immediate loading can be defined as taking place up to 7 days after implantation.[16] There is also disagreement with regard to the occlusal contact of crowns or other prosthetic restoration. Some authors claim that prosthetic restorations loaded immediately can be used without occlusal contacts, while others claim that the term "immediate loading" applies only to prosthetic restorations that are in full occlusion, both in centric and eccentric movements.[17–19] However, there is agreement that the most important criterion for applying the immediate loading protocol is that the implant achieves adequate primary stabilization. This is possible due to a high value of insertion torque, which seems to be crucial in the immediate loading procedure. On the basis of many studies identified in Esposito et al.'s review, this value was determined to be above 35 N·cm.[16]

Primary stabilization is the mechanical anchoring of the implant to bone. It enables the correct process of osseointegration, i.e., the direct functional and

Table 2. Characteristics of the selected studies

Author	Study design	Follow-up period	Type of loading	MBL [mm]
Al Amri et al. 2016[5]	30 healthy people	2 years	immediate	0.46 ±0.16
	30 T2D patients (HbA1c: 6.1–8.0%)			0.58 ±0.15
	31 T2D patients (HbA1c: 8.1–10.0%)			0.59 ±0.20
Bilhan et al. 2010[6]	252 implants in 87 patients	3 years	early (60–90 days)	1.01 ±0.15
			late (91–140 days)	0.90 ±0.18
			late (>140 days)	1.07 ±0.13
Crespi et al. 2007[7]	160 implants in 27 patients	18 months	immediate, distal side, maxilla	0.84 ±0.69
			immediate, distal side, mandible	1.24 ±0.60
Galli et al. 2008[8]	104 implants in 52 patients	14 months	immediate	1.10 ±0.58
			early	1.11 ±0.54
Gjelvold et al. 2021[9]	2 implants	12 months	immediate (smokers)	1.86 ±1.33
	5 implants		late (smokers)	0.91 ±0.66
	22 implants		immediate (non-smokers)	0.37 ±0.55
	18 implants		late (non-smokers)	0.44 ±0.62
Krawiec et al. 2021[10]	40 implants	12 months	early (within 4 weeks)	0.14 ±0.24
Krawiec et al. 2021[11]	40 implants	12 months	early (within 4 weeks)	0.20 ±0.88
Meijer and Raghoebar 2020[12]	15 implants in 15 patients	12 months	immediate	0.17 ±0.73
Pellicer-Chover et al. 2016[13]	10 crestal implants	12 months	early	0.06 ±1.11
	13 subcrestal implants			1.22 ±1.06
Zöllner et al. 2008[14]	383 implants	3 years	total	0.70 ±0.83
			immediate	0.56 ±0.73
			early	0.82 ±0.89

T2D – type 2 diabetes; HbA1c – glycated hemoglobin.

structural connection of bone tissue with the implant surface.[20,21] Proper primary stabilization is a condition of the implant when movements are sufficiently reduced. Micromovements cannot be completely eliminated, but can be reduced by binding implants together (for example in a bridge), and also by removing lateral contacts, thus diminishing lateral forces.[22] Unless primary stabilization is achieved, healing is disturbed by inhibiting osteoblasts. Consequently, connective tissue is formed between the implant and bone (fibrointegration), which results in the loss of the implant. This phenomenon has been studied in animal models.[23]

Over the years, many studies have been carried out on factors that may affect primary stabilization, including the following:

– the appropriate preparation of the implant bed[18];
– the use of appropriate techniques, such as osseodensification drilling[24];
– the appropriate shape and length of the implant[16];
– the appropriate modification of the implant surface[10,25];
– the type of implant and the distribution of load[26,27];
– the condition of bone tissue and its changes during treatment[28];
– demographic factors[9]; and
– the type of crown.[9]

The structure of the implant surface is relatively well examined. As previously shown, the appropriate modification of the implant surface, e.g., chemical modification through the immersion of the implant in a special solution[11] or acid etching[14], may increase the probability of achieving primary stabilization by the implant at a level that allows its immediate loading.

The use of immediate implant loading provides many benefits. First of all, it reduces treatment time, which positively affects patient satisfaction. The quick restoration of missing teeth increases patient comfort and reduces the negative psychological effects caused by tooth loss.[29] Furthermore, the second surgery is avoided to uncover the implant and insert an abutment. With the immediate insertion of a crown, it is more likely to maintain the appropriate tissue contour (especially of the gingival papilla) with a better esthetic result.[30] The risk factors for complications that may occur during immediate loading are significant malocclusion, bruxism, a previous implant loss, and any other general contraindications for conventional implantation.

Early loading

Early loading is defined as the implant-prosthetic restoration carried out up to 2–3 months after implantation; however, the average time is 3–6 weeks. This period is critical due to a decline in primary stabilization and still incomplete secondary stabilization.[15,16,31] Nevertheless, during this time, the primary bone tissue is formed, the mechanical properties of which enable prosthetic reconstruction. Therefore, it seems reasonable to load the implant 3–4 weeks after implantation. Furthermore, implants with a modified surface achieve secondary stabilization faster than conventional implants.[32]

The immediate and early loading of implants have many positive aspects, e.g., fewer surgical interventions, the reduction of soft and hard tissue loss, or the shortening of total treatment time. Those benefits enable basic functions, such as eating, chewing and articulating, to be resumed faster.[33] The use of early or immediate loading is of importance, especially for the esthetic result. This applies to the area between the second right premolar and the second left premolar, which greatly affects the patient's appearance and well-being.

Late loading

The term "late loading" is used when the prosthetic structure is attached late, after the conventional healing period, which lasts at least 3–6 months.[6,31] This is a 3-step procedure. In the 1st stage, the implant site is allowed to fully heal before loading. During this phase, the osseointegration process occurs. After 3–6 months, the 2nd stage takes place, i.e., the exposure of the implant and the insertion of the healing screw (abutment). The 3rd stage involves placing a prosthetic crown.

Late loading is the most common protocol. One of its advantages is a reduced risk of implant loss after loading.[16] It is recommended in situations where primary stabilization was not achieved using a minimal insertion torque above 20 N·cm, in cases with simultaneous extensive augmentation, or when the patient is not convinced of immediate loading and does not accept the associated risks.

Marginal bone loss

Marginal bone loss is a key parameter that is used to assess the proper healing and functioning of implants.[34] This parameter is important, as the gradual loss of bone around the implant may lead to its loss. Researchers report different values of MBL. Despite somewhat varying results, MBL of 2 mm is generally regarded as the maximum acceptable value. During the 1st year after implantation, an acceptable MBL value should be no more than 1.5 mm, with an increase of 0.2 mm per year in subsequent years.[1,35] Currently, however, it is claimed that no marginal bone atrophy is the only evidence of the adequate healing of the implant (the so-called "zero bone loss concept").[36] It is worth considering, especially in young patients, in whom even the 'acceptable' bone loss would amount to 6 mm 20 years after implantation.

Marginal bone loss depends on many factors. One of the most important ones is the thickness of the gingiva around the implants. The critical value is defined as 2 mm; below this value, the risk of atrophy is much higher.[37]

Other factors include:
– poor oral hygiene[5];
– the position of the implant in relation to the anatomy of the bone[13,38];
– the presence of keratinized gingiva[39,40];
– an improperly made prosthetic restoration[41];
– the type of implant[42,43];
– the type of prosthetic restoration[44];
– the type of abutment, and the type of connection between the implant and the abutment[45,46];
– bruxism[47];
– tobacco smoking[9,48];
– a history of periodontal disease[49,50];
– general diseases, e.g., diabetes[5,51]; and
– the experience of the operator and the dental technician.[52,53]

It should be noted that marginal bone atrophy is independent of gender, the degree of primary and secondary stabilization, and the crown-to-implant ratio, while the effect of age is inconclusive.[54–56]

To assess the MBL value, cone-beam computed tomography (CBCT) or radiovisiography (RVG) can be used. In order to correctly determine it, 2 measurements – made on the day of implantation and during the follow-up visit – should be compared. Using the 'As Low As Reasonably Achievable' (ALARA) principle, to minimize the radiation dose, 2 RVG (2–5 microSv) images taken using the right angle technique are sufficient to measure MBL. This ensures the repeatability of the images, and thus the correctness of the measurements.[57]

General considerations

Receiving an answer to the question of whether immediate or early implant loading have important clinical implications as compared to the conventional treatment is important for the physician and the patient, since it enables faster restoration of the functions of the stomatognathic system. Therefore, in recent years, scientists have searched for ways of shortening the time of treatment without affecting its quality. Much research has been conducted to assess the effect of loading time on MBL. A meta-analysis by Esposito et al. showed that there were no significant differences between immediate and conventional loading.[16] In contrast, comparisons between early (6 weeks) and conventional loading as well as between early and immediate loading provide insufficient and inconclusive evidence to determine whether there are any clinically significant differences between the protocols. A meta-analysis by Suarez et al., which included 11 articles, showed that the time of implant loading did not affect MBL.[31] It was also revealed that after achieving osseointegration, there were no differences between the protocols.[31] Similar conclusions can be drawn based on other meta-analyses.[58–61]

The conclusions of the meta-analyses are in line with those of clinical trials, but the latter provide a deeper understanding of other factors that can play a role in the maintenance of proper bone tissue. A prospective controlled clinical trial conducted by De Smet et al. reported that distal implants were more likely to fail in the immediate loading protocol.[62] According to Kawai and Taylor, the immediately loaded implants showed a loss of about 0.6 mm in the first 12 months, and the same or greater value in the 2nd year.[63] Conversely, the implants receiving conventional loading showed almost the same loss as the immediately loaded implants in the 1st year, but resulted in less MBL in the following year.[63] Aires and Berger[64] as well as Crespi et al.[7] claim that the loading of implants immediately after extraction can be carried out successfully. Bilhan et al.[6] and Sommer et al.[29] indicate that late loading increases the risk of MBL. This is due to the lack of occlusal forces in this protocol, which act on the implant and activate proper bone remodeling. However, in some reports, immediate or early implant loading may be associated with slightly greater MBL, and also with a higher overall risk of implant failure.[4,65] Discrepancies in the results may be due to many reasons.[19,65] Possible factors include research bias, a small number of studies available in the literature, small samples sizes, short observation periods, ambiguous definitions of implant loading time, the types of implants and prosthetic reconstruction used, the characteristics of missing teeth (single, multiple, in the mandible, in the maxilla, anterior or lateral), the previously conducted augmentation procedures, whether the reconstruction is made in occlusion or not, etc. Thus, more well-designed and randomized controlled trials (RCTs) in line with the CONSORT (Consolidated Standards of Reporting Trials) guidelines are needed.[16] It is also important that the treating physician understands the healing processes of bone and soft tissue, and performs a thorough examination of the patient's characteristics, which allows selecting the appropriate therapy and avoiding possible complications.

Conclusions

The analysis of the available data leads to the conclusion that there are no statistically significant differences in the occurrence of marginal bone atrophy between the immediately and early loaded implants as compared to the conventionally loaded ones. Nevertheless, immediate and early implant loading are important alternatives for properly selected and compliant patients undergoing treatment according to the guidelines. Further studies are needed to determine other factors, in addition to the type of protocol, to ensure better patient satisfaction .

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

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Review

Oral manifestations of monkeypox: Brief review

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Abstract

The current outbreak of monkeypox infection has caught the attention of people worldwide. Updated data showed a dramatic increase in the number of patients in many non-endemic countries. An emergence of monkeypox together with coronavirus disease 2019 (COVID-19) caused a tremendous burden on the healthcare system, globally.

The aim of this review was to briefly describe the current situation, transmission, clinical features, diagnosis, and prevention of the disease. Oral manifestations of monkeypox as well as those of similar viral diseases were elaborately delineated.

The outbreak of monkeypox in non-endemic regions has expanded to at least 47 countries with more than 4,100 new infections. The clinical features in non-endemic regions are atypical and different from those in central and western Africa. Milder symptoms with no death cases have been observed. The oral mucosa is often involved and oral lesions may initially be manifested before the rash spreads to the face and other parts of the body. The diagnosis of monkeypox is mainly based on clinical presentations and laboratory investigations. Prevention by avoiding close contacts with patients and sick animals and providing vaccination to those who have a primary contact with patients is essential.

Oral manifestations may occur prior to skin eruptions, suggesting that dentists and dental personnel should be well aware of the nature of the disease. Prevention and public awareness of the disease are crucial for mitigating further human-to-human transmission.

Keywords: infection, outbreak, monkeypox, oral manifestations

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Introduction

Monkeypox is a rare zoonotic disease caused by the monkeypox virus.[1,2] The monkeypox virus, as well as the smallpox virus, belongs to the Orthopoxvirus genus in the family Poxviridae. The first human case of monkeypox was recorded in 1970 in the Democratic Republic of the Congo. Since then, monkeypox has become an endemic disease in western and central Africa. Until recently, the outbreak of the disease has been reported in 47 non-endemic countries in Europe, North and South America, Asia, north Africa, and Australia.[3] As of June 24, 2022, more than 4,100 confirmed cases have been reported. Notably, 87% of all cases have occurred in European countries.[4] Although the main cause of the new outbreak remains unclear, it is believed that the cessation of smallpox vaccination, which provided some cross-protection against monkeypox, resulted in an increased human-to-human transmission.[2]

The objective of this review was to briefly report the updated information on the outbreak, transmission, clinical features, oral manifestations, diagnosis, and prevention of monkeypox. Oral manifestations of monkeypox and other viral infections, as well as the zoonotic nature of the disease and other related viral infectious diseases were delineated and discussed.

Methods

The PubMed database and Google Search engine were used to achieve updated and relevant data on monkeypox from 2004 to 2022. The key words were "monkeypox", "outbreak", "oral lesions", and "oral manifestations". Only relevant articles written in English were chosen. The most recent reports of the World Health Organization (WHO), Centers for Disease Control and Prevention (CDC) and Public Health England were also thoroughly analyzed. Hand searches were performed on viral zoonotic diseases and oral manifestations of viral infectious diseases including herpes simplex virus (HSV), herpes zoster, chickenpox, measles, hand-foot-and-mouth disease, human papillomavirus (HPV), and coronavirus disease 2019 (COVID-19). The exclusion criteria were articles without reliable sources or poorly written articles.

Results

Twenty-six relevant articles/reports were taken for consideration and used for the review of the disease's transmission, clinical features, oral manifestations, diagnosis, and prevention. Additionally, 11 hand-searched articles were used for the discussion.

Transmission

In general, human-to-human transmission occurs through direct physical contact with ulcerated skin or mucosa, respiratory droplets or contact with contaminated materials such as linens, bedding, electronics, and clothing.[5,6] Pregnant women can pass the virus to fetuses via the placenta.[7] Airborne transmission of monkeypox virus remains a controversial issue. If it does occur, it may not be the main route of transmission.

Alarmingly, most cases in non-endemic regions are associated with men who have sex with men (MSM), aged between 20–50. For example, all cases reported in Italy were MSM who had multiple sexual partners or practiced unprotected sexual intercourse.[8] These data suggest a human-to-human transmission of monkeypox infection through sexual contact.[8,9] However, more data are required to define monkeypox as a sexually transmitted disease. This unexpected pattern of transmission is believed to occur by a coincidental introduction of monkeypox into MSM networks and when the disease has remained circulating there.[9] The latest press briefing from WHO reported that cases from Germany and Italy showed monkeypox DNA in some patients' semen. These findings raise a probability of sexually transmitted nature of the disease.[10]

Clinical features

Historically, monkeypox infection begins with a non-contagious incubation period, ranging between 7–14 days or 5–21 days.[1] During this period, patients remain asymptomatic. Notably, the symptoms of patients with monkeypox in the endemic regions are more severe and cause a certain number of fatalities, while lesions in patients in non-endemic regions are more localized and have a different distribution of rashes. So far, there is no death report in patients from the non-endemic regions.

Monkeypox begins with the prodrome: fever, chill, headache, back pain, myalgia, asthenia, and lymphadenopathy.[1] Infected patients may be contagious during the prodromal period.[11] It is of interest to note that the main difference between symptoms of smallpox and monkeypox is that monkeypox causes lymphadenopathy while smallpox does not. Involved lymph nodes in monkeypox include submandibular, cervical, axillary, and inguinal lymph nodes and may be bilateral or unilateral. Following the prodrome, lesions may first develop in the mouth and/or oropharynx prior to the skin.[7,11] The skin rash tends to be more concentrated on the face and extremities, including palms of the hands and soles of the feet.[12,13] The lesions progress through several stages: macule, papule, vesicle, pustule, and crust. Patients are considered contagious until all the lesions become crusted.[14] The lesions are often described as painful and later itchy. Scars with hyper/hypopigmentation could occur after scabs have fallen off. The illness typically lasts for 2–4 weeks.

The signs and symptoms of patients in the new outbreak, non-endemic areas are atypical and quite different from those in western and central Africa. These include genital, perianal and perioral/oral rash, fever, lymphadenopathy, and pain when swallowing.[6,8] The lesions on the anogenital area and oral mucosa may appear first before or without spreading to the other parts of the body, suggesting sexual contacts as the route of transmission.[3,15] Several patients developed pustules before having fever. Some patients who have only few localized skin lesions may not even have any pain symptoms. Interestingly, lesions at different stages may occur in the same individuals. Lesions in the anus and rectum can cause pain, bleeding, proctitis, and tenesmus.[3] These symptoms have never before been described in patients in the endemic region. In all, the symptoms of patients in the current outbreak regions are milder than those in the endemic region. Few hospitalizations have been reported and the main reasons for hospitalization were pain management and treatment of secondary infections.

Oral manifestations

Oral and oropharyngeal lesions of monkeypox can appear as the first lesions prior to skin rashes.[3,7,11] It is described that oral sore in patients with monkeypox is a common feature in combination with fever and swollen lymph nodes.[6,16] Notably, the CDC reported that 70% of individuals had lesions in the mouth and on the tongue.[17] These data suggest that saliva can harbor the virus and the transmission can occur through oral–skin and/or oral–anogenital contact.[18] Therefore, it is important for dentists and dental personnel to recognize and be aware of the oral lesions of monkeypox. The development of the oral lesions should be similar to that of the skin lesions, starting from macule, papule, vesicle, and pustule.[19] After breaking off the roofs of the vesicle or pustule, ulceration with pseudomembrane takes place. In a recent case in the US, a male patient as a returning traveler from Nigeria developed right cervical lymphadenopathy, numerous 2–4-mm pustules with central umbilication of the skin, particularly the face, neck and hands.[20] The oral lesions were described as a few round 2–3-mm erosions on the mucosa, suggesting that the initial vesicles or pustules have already broken off, and an intact pustule on the lower labial mucosa. Main symptoms and oral manifestations of monkeypox are shown in Table 1.[11,12,19,20]

Diagnosis

Monkeypox shares similar features of the mucocutaneous lesions with many diseases including chickenpox, measles, bacterial skin infections, scabies, syphilis, and medication-induced allergies.[12] Sometimes, it is difficult to distinguish between these diseases based only on the clinical presentation. Lymphadenopathy during the prodromal stage can be used to distinguish monkeypox from chickenpox or smallpox. If monkeypox is suspected, healthcare providers should collect a sample for further polymerase chain reaction (PCR), a technique that provides good specificity and sensitivity for the detection of monkeypox virus.[21] Samples should be taken from the roof or fluid from vesicles, pustules or dry crusts, stored in a dry, sterile tube without viral transport media, and kept cold. Biopsy is an optional means for obtaining the diagnosis.

Since the beginning of the COVID-19 pandemic, the biosample collected by a nasopharyngeal swab has been used as the gold standard for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Recently, the saliva has emerged as a convenient and cost-effective biofluid for COVID-19 diagnostics and may eventually replace a nasopharyngeal swab.[22,23] The collection of the saliva is non-invasive, inexpensive and uses a simple technique without the need for special equipment. Since monkeypox infection often manifests in the oral cavity, the saliva in patients may harbor the virions of monkeypox and, therefore, could potentially be used as the biosample to detect the virus. Further investigation to validate the use of the saliva for monkeypox diagnostics will warrant great benefits for those patients.

Prevention

The measures to prevent monkeypox infection include: (1) avoiding contact with animals that could harbor the virus; (2) avoiding contact with any materials that have been in contact with sick animals; (3) isolating patients with monkeypox from others; (4) practicing good hand hygiene after contact with both infected animals or humans; and (5) using personal protective equipment (PPE) when treating patients.[7]

Table 1. Symptoms of monkeypox

Symptoms	Description	Distribution	Stages
Prodrome	duration: 5 days; fever, chill, intense headache, back pain, myalgia, intense asthenia, lymphadenopathy (bilateral/unilateral)	–	–
Skin lesions	begin within 1–3 days of appearance of fever	face, extremities, trunk, hand palms, foot soles, conjunctivae, corneas, genitalia, anorectum	macule, papule, vesicle, pustule, crust
Oral/oropharyngeal lesions	begin prior to skin eruption	lips, tongue, oral mucosa, oropharynx	macule, papule, vesicle, pustule, ulceration

Vaccination against smallpox provides 85% protection for monkeypox and prevents severe complications in infected patients.[24,25] Since the latest outbreak of monkeypox in the US, the U.S. Food and Drug Administration (FDA) has approved JYNNEOS™ (also known as Imvamune or Imvanex) for the prevention of monkeypox in individuals aged over 18.[26]

Discussion

It is of interest that a few viral infections in humans are zoonotic diseases, including monkeypox, acquired immunodeficiency syndrome (AIDS) and COVID-19. Monkeypox was first reported in 1959 as an outbreak of a pox-like disease in monkeys kept at a research institute in Copenhagen, Denmark.[27] The first human case was reported in 1970. Since then, human-to-human transmission has dramatically increased in central and western Africa. Recently, the disease has been imported to and spread out in several non-endemic countries. Acquired immunodeficiency syndrome is caused by 2 lentiviruses, namely human immunodeficiency virus type 1 (HIV-1) and human immunodeficiency virus type 2 (HIV-2). Previous data has shown that HIV-1 and HIV-2 are the result of multiple cross-species transmissions of simian immunodeficiency viruses, naturally infecting African primates.[28] Another possible zoonotic viral disease is COVID-19, caused by SARS-CoV-2. It is believed that SARS-CoV-2 emerged as a recombinant virus between the bat coronavirus and a coronavirus of unknown origin.[29] The resulting recombination of these coronaviruses enhances viral capability to cross the animal-to-human species barrier. Collectively, the zoonotic diseases such as monkeypox, AIDS and COVID-19 have shown the first animal-to-human transmission and subsequent human-to-human transmissions, and have been the cause of devastating morbidity and mortality throughout the world. The fundamental etiology of cross-species transmission of these diseases, however, remains unclear. The surveillance of any possible zoonotic diseases that may happen in the future and the etiology of animal-to-human and human-to-human transmissions are yet to be closely performed for the benefit of humankind.

Oral manifestations of monkeypox are described as vesicular or pustular lesions. After the rupture of the vesicle or pustule, the ulceration takes place. The lesions in patients with monkeypox may resemble those of viral infections that involve the oral cavity, including HSV infection, herpes zoster, chickenpox, measles, hand-foot-and-mouth disease, HPV infection, and COVID-19. Oral lesions in HSV infection are mainly vesiculobullous lesions.[30] The vesicles/bullae easily break up, developing into multiple painful, shallow ulcers on the lips and oral mucosa. Recurrent lesions are more localized and often involve the lip vermilion and keratinized oral mucosa. The varicella-zoster virus causes chickenpox as a primary form in children and herpes zoster as a secondary form in adults. Herpes zoster presents with multiple unilateral vesicular eruptions on the skin and oral mucosa in the areas innervated by the affected nerves.[31] A wide variety of oral lesions can be observed in patients with measles. These lesions include tiny, white plaque-like papules (Koplik's spots), ulcerations and necrotizing gingivostomatitis.[32] Hand-foot-and-mouth disease, mainly affecting children younger than 10 years, causes maculopapular or papulovesicular rashes on the hands and soles of the feet, as well as painful oral ulcerations.[33] Various HPV-associated diseases in the oral cavity, such as squamous papilloma, verruca vulgaris, condyloma acuminatum, Heck's disease, leukoplakia, lichen planus, and squamous cell carcinoma, do not produce oral vesicles or ulcerations but mainly keratinized verrucopapillary lesions.[34]

Currently, COVID-19 remains an overwhelming pandemic throughout the globe. Coincidentally, monkeypox has become spreading out in many non-endemic regions. The diseases cause a great burden on the global healthcare system. Since oral manifestations of monkeypox may mimic those of COVID-19, especially the vesiculobullous lesions and ulcerations (Table 2),[19,20,35,36] dental health professionals should be able to recognize and differentiate between the clinical features and oral manifestations of monkeypox and COVID-19, as well as oral viral infectious diseases mentioned above. In addition, oral manifestations following COVID-19 vaccination, particularly aphthous-like ulcers should also be included in the differential diagnosis.[37]

Conclusions

Monkeypox has become an emerging infectious disease that is increasingly spreading to many parts of the world. Healthcare providers should be aware of the nature of the disease. Oral lesions may present as initial lesions, suggesting that dentists and dental personnel are the first

Table 2. Table 2. Oral manifestations of monkeypox and COVID-19

Disease	Oral manifestations
Monkeypox	painful vesicular, pustular or ulcerated lesions occur on the lips, tongue, oral mucosa, and oropharynx
COVID-19	various types of lesions/symptoms include vesiculobullous lesions, candidiasis, ulcerations due to herpes simplex viral infection, non-specific ulcerations, transient lingual papillitis, glossitis, geographic tongue, petechiae, aphthous stomatitis, mucositis, pharyngitis, gingivitis, desquamative gingivitis, dysgeusia, xerostomia, oral pain, Kawasaki-like disease

COVID-19 – coronavirus disease 2019.

who may encounter monkeypox infection. It is important to note that recognizing and identifying oral lesions in patients with monkeypox leads not only to providing further investigation and appropriate care but also to preventing cross-infection among health personnel and patients.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

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