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Reviews

A step forward with a new vision

Mieszko Więckiewicz and Helena Martynowicz
Editors-in-Chief of *Dental and Medical Problems*

"What we know is a drop, what we don't know is an ocean."
Isaac Newton

We feel extremely honored and privileged to have been appointed as Editors-in-Chief of *Dental and Medical Problems* (DMP).

Firstly, we would like to focus on the future of DMP with regard to difficult pandemic times. The COVID-19 pandemic has given rise to new social mores, fears, and also challenges in research and medical care.[1,2] We will do our best to ensure that DMP meets all these demands. We strongly believe in the power of interdisciplinarity in science, and therefore, DMP will be the major forum for research on the spectrum of both dentistry and medicine.

According to WHO, health refers to the state of complete physical, mental and social well-being, and not merely the absence of disease or infirmity.[3] Considering this definition, DMP will offer a wide spectrum of new editorial sections, including cardiology, internal medicine, pain, sleep medicine, genetics, and many others. Our previous Editor-in-Chief Prof. Tomasz Konopka, who served for the past 17 years, as well his team deserve gratitude and respect for their work and for bringing recognition and honor to DMP. However, *panta rhei* and medicine has been changing. Modern disciplines, such as telemedicine and laser therapy, have to find their place in DMP.

Our primary aim is to maintain the level of prestige and improve the indexation and impact factor of the journal. The new editorial team, comprising excellent researchers and clinicians, will strive to attract the best articles both in medicine and dentistry. We are proud to work with a new international advisory board that includes eminent scientists from the USA, Israel, Canada, Italy, Germany, Turkey, Japan, Saudi Arabia, Spain, Australia, the Netherlands, and Poland. The new editorial team will strongly commit to make the best research available to our Readers and further improve the scientific quality in the next few years. We hope that DMP is entering a new phase of development, toward a more interdisciplinary profile, while standing as a distinctive international journal. We have planned to take several actions to speed up the article review process of our journal and make it more interesting to Authors. Moreover, we have decided to use social media for disseminating the DMP content to increase the number of citations of articles and their impact.[4]

We conclude this editorial by requesting respectable researchers to continue to support DMP by submitting their works for publication and playing an active role in the review process. We also invite interested people to contact us with any creative suggestions and ideas for improving our journal.

On behalf of the new editorial team, we take the privilege to welcome the Readers to the first issue of 2021. Enjoy reading.

Mieszko Więckiewicz
Editor-in-Chief

Helena Martynowicz
Editor-in-Chief

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Original papers

Oral manifestations in mild-to-moderate cases of COVID-19 viral infection in the adult population

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

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Abstract

Background. Coronavirus disease 2019 (COVID-19) is a serious worldwide threat presented by a broad range of symptoms, from mild flu to severe pneumonia. A rising number of atypical infections have been reported. Thus, scientists and clinicians are doing hard work to unravel scientific knowledge about this novel pandemic.

Objectives. The aim of the present work was to highlight the oral manifestations which could be observed in mild-to-moderate cases of COVID-19.

Material and methods. A questionnaire survey was performed on 665 Egyptian patients who were confirmed COVID-19-positive based on the polymerase chain reaction (PCR) test. After applying the exclusion criteria, cases with mild-to-moderate symptoms were included in the study. The questionnaire consisted of 4 sections. The 1st section included demographic data, smoking, alcohol consumption, and general health status. The 2nd section contained questions regarding the oral hygiene status of the patients, and additionally a question about the hygienic measures they took while being infected with COVID-19. The 3rd section included questions about the most commonly reported COVID-19 symptoms the patients suffered from. The 4th section included questions that referred to the oral manifestations the patients complained of while being infected with COVID-19.

Results. A total of 573 patients were included in this survey. It was reported that 71.7% of COVID-19 patients presented with some oral manifestations at a level of significance, with variable incidence – oral or dental pain (23%), pain in jaw bones or joint (12.0%), halitosis (10.5%), ulcerations (20.4%), and xerostomia (47.6%). Some patients (28.3%) showed 2 or 3 manifestations simultaneously.

Conclusions. It was proven that mild-to-moderate cases of COVID-19 infection are associated with oral symptoms, and thus the significance of dental examination of patients with communicable diseases should be emphasized.

Key words: xerostomia, oral hygiene, halitosis, coronavirus, COVID-19

Cite as
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Introduction

Coronavirus disease 2019 (COVID-19) is a serious worldwide threat caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] In COVID-19, initially lungs are infected, and patients can present with a broad range of symptoms, from mild flu to severe pneumonia and possibly fatal respiratory illness.[2]

In COVID-19 infection, the most commonly reported symptoms are a headache, a sore throat, diarrhea, the loss of taste and smell, and shortness of breath.[3] Dermatological manifestations have also been observed, and few authors have reported oral signs and symptoms.[4] It is not strange that COVID-19 can manifest itself through oral symptoms, since various pulmonary diseases and systemic disorders with pulmonary involvement are associated with oral manifestations.[5]

Meticulous checkups of the oral cavity in respiratory medicine can help in clinical diagnosis, and in providing advice and guidance to patients. In addition, dentists who detect atypical oral lesions should seek appropriate guidance and professional medical advice.[5]

Objectives

The main objective of this study was to highlight the oral manifestations which could be reported in mild-to-moderate cases of COVID-19. Moreover, our goal was to spread awareness among physicians, general and dental practitioners, about these oral symptoms so that early diagnosis could be achieved, thus maintaining patient's ultimate health and welfare.

Subjects and methods

Study design

An online questionnaire was designed for this investigation, because nearly all available questionnaires did not address the various possible oral manifestations of COVID-19 infection. A total of 665 Egyptian patients who recovered from COVID-19 were recruited from several Ministry of Health and university hospitals, including recovered dentists, nurses and physicians, who voluntarily participated in answering the questionnaire. A sharable link on Google Drive was sent to each patient via a WhatsApp message. A digital consent to participate in this study was obtained before completing the questionnaire. A web-based survey tool (Google Forms) was used to design the questionnaire.

Ethical statement

The current study protocol was approved by the ethics committee of the Faculty of Dentistry at Cairo University in Egypt (approval No. 21/6/20).

Exclusion criteria

The following exclusion criteria were adopted: failure to complete the whole questionnaire; patients with poor oral hygiene or suffering from any of the oral symptoms investigated before the pandemic; patients with chronic illnesses; smokers; alcoholics; patients with serious COVID-19 infection, who experienced severe respiratory failure (severe pneumonia, severe dyspnea, an increased respiratory rate of >30 breaths/min, and a decreased oxygen saturation of <93%) or patients who required hospitalization.

Inclusion criteria

The inclusion criteria were as follows: Egyptians; adults, 18–50 years old; laboratory-confirmed COVID-19 infection (the polymerase chain reaction (PCR) test); non-smokers; non-alcoholics; medically free patients; patients with mild-to-moderate symptoms; patients with good oral hygiene and not suffering from any oral manifestations before the pandemic.

Questionnaire tool

The questionnaire consisted of 4 sections and a total of 38 questions (Table 1). The 1st section included demographic data as well as smoking, alcohol consumption and general health status. The 2nd section contained questions regarding the oral hygiene status of the patients; it was designed in accordance with Kamel et al.'s questionnaire,[6] in addition to the WHO oral health surveys.[7] Furthermore, a question about the participants' hygienic measures taken while being infected with COVID-19 was added to the same section. The 3rd section included questions about the most commonly reported COVID-19 symptoms the patients suffered from. The 4th section included questions referring to the oral manifestations the patients complained of while being infected with COVID-19.

Assessment of the oral hygiene status

The oral hygiene of the patients was measured based on their answers from the 2nd section of the questionnaire, where the best answer for each question was given score '1', while '0' was given to other choices. The full score was 12; the participant's oral hygiene was considered good when at least 75% of this score was obtained.[6,7]

Table 1. Questionnaire for investigating the oral manifestations of coronavirus disease 19 (COVID-19)

Questions	Answers	
Do you agree to participate in this study?	yes	no
1st section: Demographic data		
Gender	male	female
Age		
Nationality	Egyptian	other
Level of education		
Chronic illnesses	yes	no
Smoking	yes	no
Alcohol consumption	yes	no
2nd section: Oral hygiene status and the estimation of the rate of applying hygiene measures		
1. How many times during the day do you brush your teeth?	twice a day or more frequently	once a day or less frequently
2. How do you clean your teeth?	a toothbrush, fluoride toothpaste and dental floss	a toothbrush only
3. How often do you change your toothbrush?	once in 3 months	once in 6 months or less frequently
4. Do you use mouthwashes containing fluoride?	often	rarely or never
5. Do you complain of bleeding on brushing or gingival bleeding?	rarely or never	at every brushing or almost at every brushing
6. Do you suffer from tooth sensitivity?	rarely or never	often
7. How often do you visit a dental clinic for a checkup?	once a year or more frequently	once every few years or in case of pain
8. Do you have calculus regularly removed?	once every few years, once a year or more frequently	rarely or never
9. Are there any cavities in your teeth?	no cavities at all / don't know / / 1–2 cavities	3 cavities or more
10. Do you have teeth that are mobile?	no / I don't know	yes
11. Did you suffer from any of the following before the epidemic? bad smell / ulcerations / dry mouth / oral or dental pain / pain in jaw bones or joint	yes	no
12. What were your oral hygiene measures while being infected with COVID-19?	increased	the same / decreased
3rd section: COVID-19 symptoms		
1. Was your PCR test result for COVID-19 positive?	yes	no
2. Did you complain of fever?	yes	no
3. Did you complain of cough?	yes	no
4. Did you complain of a sore throat?	yes	no
5. Did you complain of malaise?	yes	no
6. Did you complain of a headache?	yes	no
7. Did you complain of diarrhea?	yes	no
8. Did you complain of the loss of smell sensation?	yes	no
9. Did you complain of the loss of taste sensation?	yes	no
10. Did you complain of muscle pain?	yes	no
11. Did you experience dyspnea or shortness of breath?	yes	no
12. Did you need hospitalization?	yes	no
4th section: Oral manifestations		
1. Did you complain of any oral manifestations while being infected with COVID-19?	yes	no
2. If yes, which of the following did you complain of?		
Halitosis (bad smell)	yes	no
Xerostomia (dry mouth)	yes	no
Ulcerations	yes	no
Oral or dental pain	yes	no
Pain in jaw bones or joint	yes	no

PCR – polymerase chain reaction.

Statistical analysis

Data was coded and entered using the statistical package IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA). Categorical variables were summarized as frequency and percentage. Quantitative variables were summarized as mean (*M*) and standard deviation (*SD*). Comparisons between the groups, regarding the incidence of the studied parameters, were made using the χ^2 test. A *p*-value <0.05 was considered statistically significant.

Results

Survey respondents

A total of 665 patients agreed to take part in the questionnaire. Ninety-two patients were excluded from the analysis: 9 were under 18 years old; 11 did not have a confirmed positive PCR test result; 3 had Sjögren's syndrome; 13 were hospitalized; 6 did not complete the questionnaire; 7 were smokers; 21 had chronic illnesses; 9 were not Egyptians; and 13 had poor oral hygiene. Therefore, the final analysis was performed on 573 patients. Overall, the respondents submitted the questionnaires from May 1, 2020 to July 1, 2020.

Demographic data

The mean age of patients was 36.19 ±9.11 years (range: 19–50 years). There were 408 females and 165 males. Regarding the educational level, patients were categorized into 3 levels: elementary education (n = 75); bachelor's degree (n = 444); and postgraduate education (n = 54).

Analysis of COVID-19 symptoms

In this study, we mainly included patients with mild-to-moderate symptoms, without severe respiratory failure, who were not hospitalized. As reported in Fig. 1, the percentage of the respondents who suffered from fever was 66.0%, cough – 55.5%, a sore throat – 52.4%, malaise – 72.8%, a headache – 70%, diarrhea – 50.3%, the loss of smell – 61.8%, the loss of taste – 55.5%, muscle pain – 76.4%, and dyspnea – 51.8%.

Analysis of the oral manifestations of COVID-19 in the respondents

It was found that 71.7% of the patients presented with some oral manifestations, with variable incidence, at $p < 0.001$. Regarding oral or dental pain, it was expressed as 23.0%, pain in jaw bones or joint – 12.0%, halitosis – 10.5%, ulcerations – 20.4%, and xerostomia – 47.6%. Some respondents (28.3%) showed 2 or 3 manifestations simultaneously.

There were statistically significant differences ($p < 0.001$) between the respondents' answers regarding each symptom except for xerostomia, where $p = 0.5$ (Table 2).

The assessment of the incidence of oral manifestations in relation to the 4 investigated parameters (gender, age, the educational level, and the rate of the oral hygiene measures taken by the patient while being infected with COVID-19) was performed as follows:

– the assessment of the incidence of oral manifestations in relation to the patient's gender – the statistical analysis identified a significantly increased number of ulcerations in male patients as compared to females ($p = 0.02$); however, pain in jaw bones or joint was significantly increased in female patients as compared to males ($p = 0.006$) (Fig. 2);

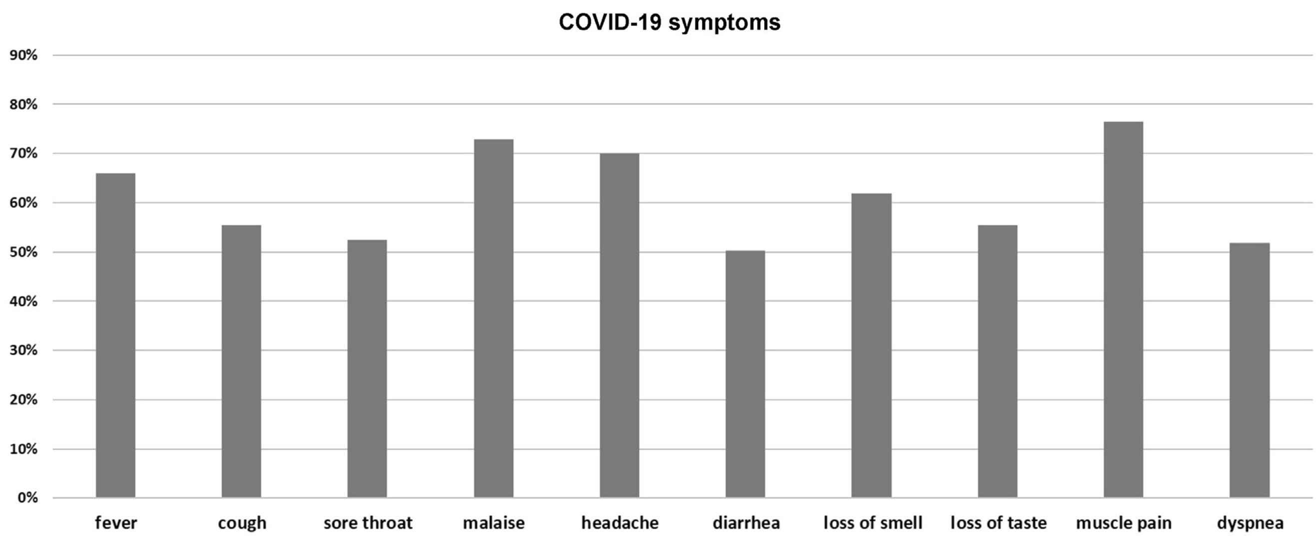

Fig. 1. Analysis of COVID-19 symptoms in mild-to-moderate cases

Table 2. Data regarding the respondents' oral manifestations while being infected with COVID-19

Oral manifestations	Answer	Number n	Percentage [%]	χ^2	p-value
Occurrence of oral manifestations	yes	411	71.7	6.00	<0.001*
	no	162	28.3		
Pain in jaw bones or joint	yes	69	12.0	110.00	<0.001*
	no	504	88.0		
Halitosis	yes	60	10.5	119.37	<0.001*
	no	513	89.5		
Ulcerations	yes	117	20.4	66.85	<0.001*
	no	456	79.6		
Xerostomia	yes	273	47.6	0.40	0.500
	no	300	52.4		
Oral or dental pain	yes	132	23.0	55.50	<0.001*
	no	441	77.0		
Combined manifestations	yes	162	28.3	36.00	<0.001*
	no	411	71.7		

* statistically significant differences between the answers ($p < 0.001$).

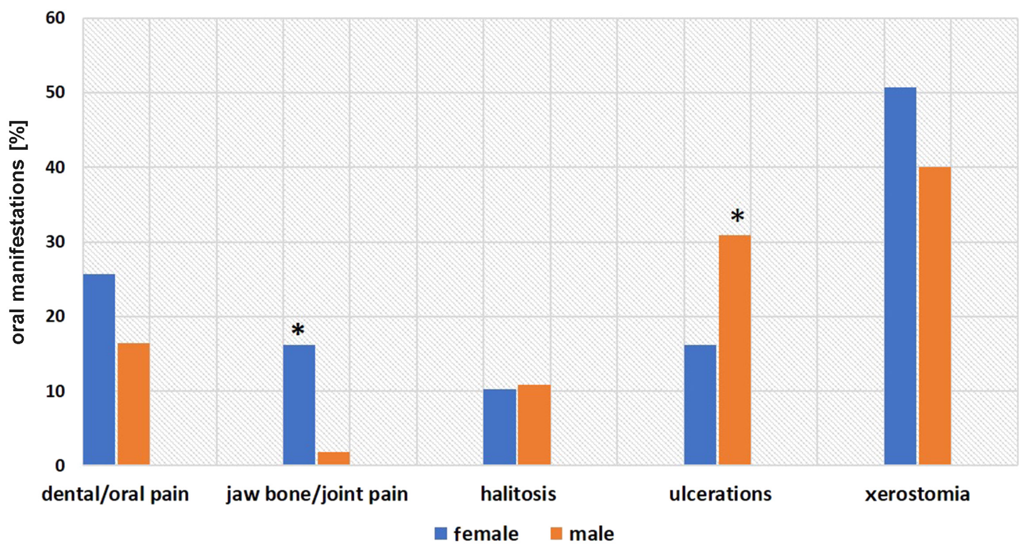

Fig. 2. Incidence of oral manifestations in relation to gender

* statistically significant differences between males and females ($p < 0.05$).

- the assessment of the incidence of oral manifestations in relation to the mean age of the respondents – the statistical analysis showed a non-significant difference in the mean age between the patients who complained of oral manifestations and those who did not ($p > 0.05$) (Fig. 3);
- the assessment of the incidence of oral manifestations in relation to the educational level of the respondents – the statistical analysis showed non-significant differences in the incidence of the oral symptoms associated with COVID-19 infection between the participants with a higher educational level and those with a lower level of education ($p > 0.05$) (Fig. 4);

Regarding the frequency of the oral hygiene measures taken while being infected with COVID-19, the patients were grouped according to their answers into 3 categories: 1 – those whose oral hygiene measures decreased while being infected (17.8%); 2 – those whose oral hygiene measures did not change (63.4%); and 3 – those whose oral hygiene measures increased while being infected (18.8%).

- the assessment of the incidence of oral manifestations in relation to the patient's oral hygiene measures taken while being infected with COVID-19 – there was a statistically significant difference between the 3 groups of patients in the incidence of oral/dental pain ($p < 0.05$) and ulcerations ($p < 0.001$), with the highest percentage of oral pain and ulcerations observed in the patients with decreased hygiene measures, and the lowest percentage of oral pain and ulcerations in the patients with increased hygiene measures (Fig. 5).

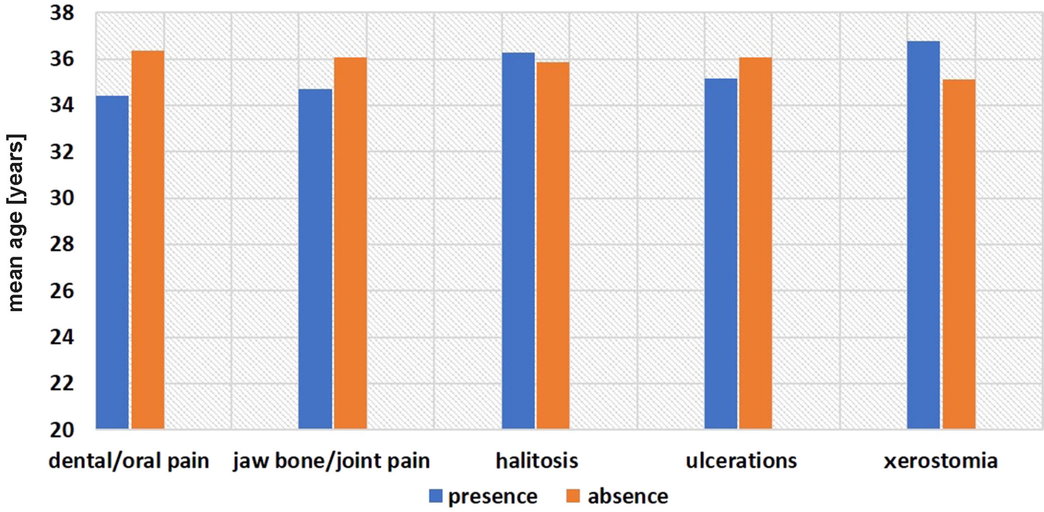

Fig. 3. Incidence of oral manifestations in relation to the mean age of the patients

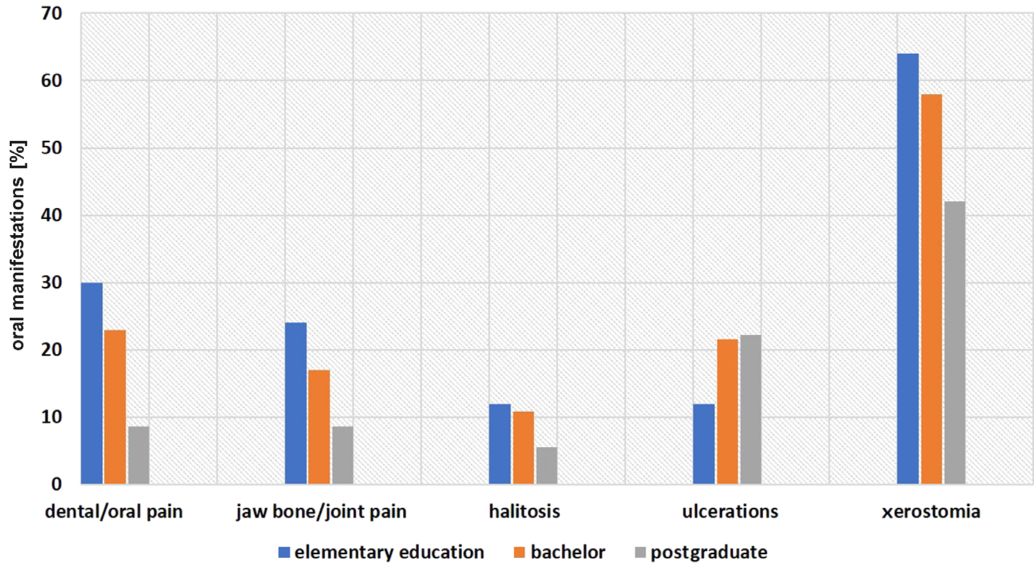

Fig. 4. Incidence of oral manifestations in relation to the educational level of the patients

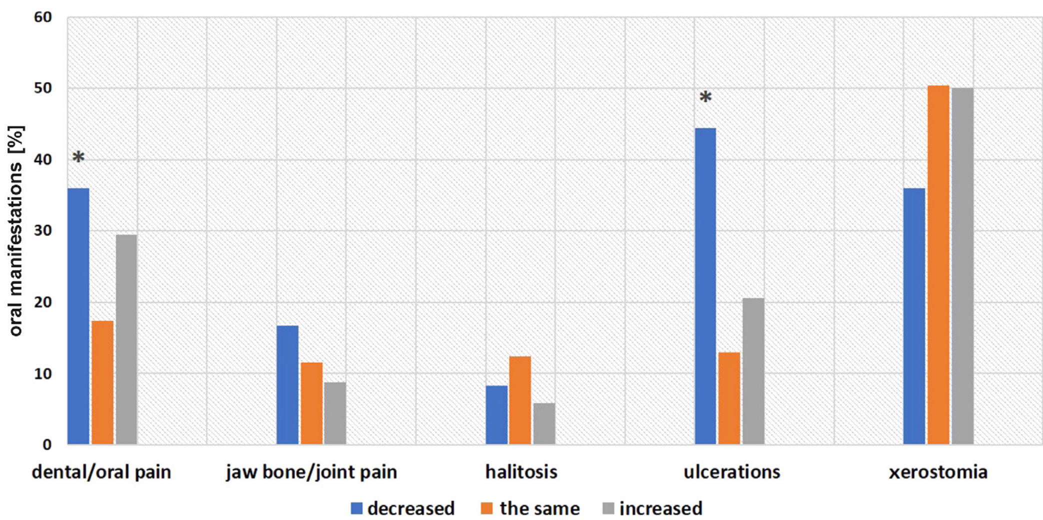

Fig. 5. Incidence of oral manifestations in relation to the rate of the hygienic measures taken by the patients while being infected with COVID-19
* statistically significant differences between groups ($p < 0.05$ for dental/oral pain and $p < 0.001$ for ulcerations).

Discussion

Clinical evidence shows that the oral mucosa is a primary site of entry for SARS-CoV-2, and is considered possibly at high risk of susceptibility to the 2019 novel coronavirus (2019-nCoV) infection. However, there is still uncertainty whether the above-mentioned oral manifestations result from direct viral infection or from systemic health deterioration and impaired immune system.[8] The aim of the present work was to investigate the oral manifestations which could be associated with mild-to-moderate cases of COVID-19 infection.

In the present work, patients experienced the general signs and symptoms of COVID-19 infection, which are fever, cough, a sore throat, malaise, a headache, diarrhea, the loss of smell, the loss of taste, muscle pain, and dyspnea. This is in accordance with several clinical studies, which reported these symptoms as the most prevalent ones in mild and moderate cases of COVID-19 infection.[9] In addition, hospitalized patients were excluded to avoid the variable of different drug usage. Moreover, a relatively narrow age range of participants was chosen, excluding subjects aged >50 years, who are considered by WHO more prone to experience severe COVID-19 symptoms.[1,3]

In the current study, 47.6% of COVID-19 patients manifested xerostomia. This is in accordance with many cases, in which respiratory tract infections were reported as the main cause of xerostomia.[10] In addition, a study involving 108 patients with confirmed COVID-19 in Wuhan reported that 46% of them complained of xerostomia.[11] In another study, 32% of patients reported dry mouth as one of their symptoms; xerostomia appeared before the onset of other general COVID-19 symptoms.[12]

It has been stated that the target of SARS-CoV-2 are angiotensin-converting enzyme 2 (ACE2)-positive cells. Angiotensin-converting enzyme 2 is detected in the duct epithelium of the salivary glands. Therefore, the salivary glands are a possible target for COVID-19 infection.[13] It is speculated that SARS-CoV-2 can enter the epithelial cells of the salivary gland duct, multiply in them and be discharged into saliva. A study by To et al. demonstrated that SARS-CoV-2 nucleic acid was found in the saliva samples of 91.7% of patients.[14] It is hypothesized that SARS-CoV-2 infection results in pathological inflammatory lesions in the salivary glands, causing their lysis at the early stages of infection; then, the salivary glands may be later destroyed by the immunopathological reactions. These findings suggest that all other oral manifestations in COVID-19 patients may develop due to a decreased salivary flow. It has also been reported that with the infection progression, amylase may enter peripheral blood after acinar cell damage.[13]

Moreover, xerostomia reported in COVID-19 patients may be triggered by stress, as dry mouth is usually found in psychiatric patients. It is commonly correlated with nutritional deficiencies, anxiety, tension, and distress, which are often observed in the case of COVID-19 patients, who are stressed because of their fear of the disease, in addition to the stressful conditions of isolation.[15,16]

In this survey, 23% of COVID-19 patients experienced oral or dental pain. This pain could be referred from muscles, as it was reported in the current study that 76.4% of COVID-19 patients experienced myalgia. It has been proven that in case of muscle pain, neurons become sensitive and the stimulus is transmitted to central nerves via neurotransmission. Subsequently, pain which originates in muscles can be felt in the upper and lower jaws.[17] Moreover, also headaches can be referred to the teeth; it was reported in the present study that 70% of COVID-19 patients suffered from headaches. In headaches, neuropeptides are released from trigeminal nerve endings and dilate blood vessels; then, the subsequent inflammation is proven to cause pain.[17] In addition, it has been reported that orofacial or dental pain sensation can be enhanced by psychological distress or emotional disturbances, which is the case in nearly all COVID-19 patients.[15,16]

Furthermore, 20.4% of COVID-19 patients reported the appearance of ulcers in their oral cavities. This is in accordance with several cases, in which blisters and oral ulcers occurred during COVID-19 infection.[8] It has been demonstrated that psychological upsets, such as anxiety and stress, contribute to the development and progression of oral lesions like recurrent aphthous ulcers, and this applies to COVID-19 patients.[16] It has also been proven that psychological distress stimulates the immunoregulatory mechanism by elevating the leukocyte count at inflammatory sites.[18] In addition, ACE2 is detected in the oral cavity and appears in high amounts in epithelial cells. It is elevated in the tongue, gingival and buccal mucosa. These findings demonstrate that the oral mucosa may be a target for COVID-19 infection.[19]

In the current study, 12% of patients complained of pain in jaw bones or joint. This is in agreement with several studies, where musculoskeletal pathologies, such as skeletal muscle, bone and joint disorders, were reported in COVID-19 patients and those with SARS-CoV-2 infection.[20,21] It has been reported that in addition to potential direct viral infection, the cytokines and pro-inflammatory signaling molecules induced by the infection can impact skeletal muscles by reducing protein synthesis and stimulating muscle fiber proteolysis. The virus SARS-CoV-2 has been proven to infect type-II pneumocytes, which line the respiratory epithelium, and express ACE2 and transmembrane protease serine 2 (TMPRSS2).[21] Angiotensin-converting enzyme 2 and TMPRSS2 are also expressed in numerous human skeletal muscle cells, several types of synovial cells, and different types of chondrocytes on articular surfaces and in the meniscus.[22]

Moreover, joint pain in COVID-19 patients is most probably caused by emotional distress rather than mechanical and occlusal aspects. Stressed people usually grind and clench their teeth. They eventually suffer from muscle fatigue and spasm. Patients experiencing joint pain usually complain of psychological symptoms, such as irritation, fear, worry, uneasiness, tension, malnutrition, and insomnia, which are the symptoms commonly experienced by COVID-19 patients.[15,16]

In the present study, 10.5% of COVID-19 patients complained of halitosis. It has been reported that oral halitosis caused by respiratory tract infections may reach up to 10%.[23] This could be due to the passage of sinus or nasal secretions into the oropharynx.[24] The pungent gases produced by different respiratory pathogens are retained in the exhaled breath, stimulate olfactory receptors, and are released through the mouth or the nose, causing malodor.[25] In addition, halitosis is also among the symptoms related to *Helicobacter pylori* infection, which is the major pathogenic cause of ulcerative alterations in the gastric mucosa. Moreover, gastroesophageal reflux disease is usually associated with halitosis.[26]

Additionally, 28.3% of COVID-19 patients in the current study showed 2 or 3 of the above-mentioned oral manifestations concurrently. This could be due to the interaction of several mechanisms in the oral cavity, where ACE2 plays the major role due to its presence in various oral tissues.[19]

The assessment of the incidence of oral manifestations in relation to the 4 investigated parameters showed that regarding gender, ulcerations were significantly more common in males than females. This is in accordance with a previous study, which showed the preponderance of ulcers among males.[27] However, this is opposite to the findings of other investigation, which reported female prevalence.[28] In the present study, it was probably hard to say what type of ulceration was present in the infected patients, since clinical examination was not applicable in those patients. However, we assumed that these ulcerations might be aphthous ulcers, as it has been reported that psychological stress plays an important role in the progression of recurrent aphthous ulcers, which is the case in nearly all COVID-19 patients, who experienced a very stressful situation.[16] On the contrary, pain in jaw bones or joint was more common in females than males. This may be due to variations in estrogen levels between both sexes, as estrogen plays an essential role in bone health. Moreover, temporomandibular joint osteoarthritis is more prevalent in females, and can cause tenderness and pain in the joint region.[29]

Regarding the mean age of the patients and their educational level, the statistical analysis showed non-significant differences in the incidence of oral symptoms in relation to these 2 measured parameters. It supports the hypothesis that these oral symptoms could result from direct viral infection and are not triggered by other variables.[8]

As for the rate of the oral hygiene measures taken by the patients while being infected with COVID-19, it was reported that in the patients with decreased oral hygiene measures, ulcerations and dental/oral pain were significantly increased. This can be attributed to plaque and calculus accumulation around the teeth in case of poor oral hygiene, which will subsequently lead to the inflammation and ulceration of the gingiva, and eventually cause periodontitis, orofacial pain and tooth loss. This finding is confirmed by a study which investigated the complications of COVID-19 in patients with poor oral health.[6,30] The authors proved that there was a link between elevated bacterial loads in the oral cavity and post-viral complications, and showed how improving oral hygiene measures could decrease the risk of COVID-19 complications.[6,30]

In the current study, there were some limitations. Firstly, we were unable to include a control group, as the questionnaire was specifically designed for COVID-19 patients. To include a healthy population, it is required to design another version of the questionnaire, with different questions to suit healthy unaffected individuals, which could lead to contrasting answers, and thus inaccurate results.

Secondly, unfortunately, the number of female participants was greater than male participants. However, in our study, we did measure several parameters other than gender. Moreover, we were concerned with the presence of oral symptoms rather than the gender effect, as there is no exact proof in the literature that gender affects the presence or absence of oral manifestations associated with viral infections.

Finally, the clinical examination of patients was not applicable in the current work, as most of the dental and university clinics were closed at the time of the present investigation, treating only emergency cases.

Conclusions

It was proven that mild-to-moderate cases of COVID-19 infection were associated with oral manifestations, with variable incidence. The most frequent oral manifestation for the included participants was xerostomia, while the least expressed symptom was halitosis.

It was found that there was a significant increase in the expression of ulcerations in male patients. However, jaw bone/joint pain was significantly increased in female patients. Moreover, a statistically significant difference in the incidence of oral manifestations in relation to the oral hygiene measures taken by the patients while being infected with COVID-19 was noticed, with the highest percentage of ulcerations and oral pain observed in patients with decreased hygiene measures. Regarding the mean age of the participants and their educational level, there were non-significant differences between the patients who complained of oral manifestations and those who did not. Based on the above, the significance of dental examination of patients with communicable diseases should be highlighted and the role of a dentist as a member of a multidisciplinary team in assisting serious cases, such as COVID-19 patients, should be emphasized.

Recommendation

Further clinical studies are recommended to figure out whether oral manifestations in COVID-19 patients are due to direct viral infection or the emotional distress and stressful conditions experienced by the patients.

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Original papers

Are individuals with orofacial pain more prone to psychological distress during the COVID-19 pandemic?

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Abstract

Background. It is of utmost importance to identify and treat groups susceptible to psychological problems during the coronavirus disease 2019 (COVID-19) pandemic.

Objectives. This study aimed to compare the psychological status between the general population and subjects with orofacial pain (OFP) during the COVID-19 pandemic.

Material and methods. A total of 509 young adults were recruited based on the inclusion and exclusion criteria, using the convenient sampling approach. They were assigned to 2 study groups: group 1 – individuals with OFP; and group 2 – the general population. Their background variables, knowledge, perception, attitude, concerns, and Kessler scale scores were recorded through an online questionnaire. Student's *t* test, the χ^2 test, Pearson's correlation coefficient, and one-way analysis of variance (ANOVA) were used for the statistical analysis.

Results. Some of the background variables were significantly different between the study groups ($p < 0.05$). The knowledge, perception and attitude scores of the respondents were not significantly different between the study groups ($p > 0.05$). Females exhibited significantly higher adjusted Kessler scores as compared to males ($p < 0.05$). Group 1 exhibited the highest adjusted Kessler scores ($p < 0.05$).

Conclusions. Individuals with OFP exhibited higher psychological distress, depressive symptoms and anxiety during the rapid rise of the COVID-19 outbreak, representing a moderate psychological disorder. Females suffered more from psychological distress as compared to males. Therefore, psychological interventions should be focused on this group.

Key words: psychological status, orofacial pain, COVID-19, Kessler scale (K10)

Introduction

The deadly coronavirus disease 2019 (COVID-19) has promptly become a pandemic with its high transmissibility.[1,2] The COVID-19 pandemic has adversely affected the mental health of health professionals, patients and the public, increasing the incidence of psychological crises.[3–6] Symptoms of adverse psychiatric outcomes have become more prevalent in different populations as compared to the era before the pandemic. Currently, varying degrees of the outbreak severity, national economy, government preparedness, the availability of medical supplies/facilities, and a proper dissemination of COVID-related information have led to regional differences in the general public's psychological health. At the beginning of the outbreak, when individuals were challenged by mandatory quarantine, unexpected unemployment and uncertainty associated with the outbreak, symptoms of adverse psychological outcomes were more commonly observed.[7]

Conditions in which an examination by the physician puts individuals at risk of contracting coronavirus disease might predispose them to psychological problems.[8] One of these conditions is orofacial pain (OFP), with a prevalence of 10–15% in the adult population.[9] After back, neck and knee pain, OFP is one of the most common causes of chronic pain.[9] Recently, it is comprehensively classified as OFP attributed to dentoalveolar disorders and anatomically related structures, myofascial OFP, temporomandibular joint (TMJ) pain, OFP attributed to the lesions or diseases of the cranial nerves, OFP resembling the presentations of primary headaches, and idiopathic OFP.[10] Acute pain in the orofacial area is often tooth-related.[9] Chronicity in OFP is defined as pain occurring on more than 15 days per month and lasting for more than 4 hours daily for at least 3 months.[10] Chronic OFP is most commonly related to musculoskeletal disorders and temporomandibular disorders (TMDs).[9] Temporomandibular disorders is an umbrella term embracing pain and dysfunction that involves the masticatory muscles, TMJ and associated structures.[9] Apart from imposing a substantial economic burden on societies,[11] persistent OFP exerts a great impact on patients' quality of life.[12] It has been suggested that, both as a confounder and a mediator, psychological distress coexists with chronic pain, and being exposed to pain might exacerbate a depressive state.[13] Comorbid psychological distress and psychosocial dysfunction have been observed in people with OFP, especially when the pain is chronic, such as in TMDs.[14–17] A study from 2005 on the presence and impact of post-traumatic stress disorder (PTSD) in a sample of patients seeking OFP treatment suggested that PTSD was prevalent in the OFP setting.[18] In the era of the COVID-19 pandemic, patients with OFP might even suffer more from psychological distress, as the disease, the lockdown of cities and the associated sequelae have increased the incidence of psychological crises in the whole world population.[3–6]

Psychological factors associated with the pandemic might even lead to a greater risk of developing and perpetuating bruxism and TMDs.[19] Also, depressive symptoms can influence the treatment outcome of OFP.[13,20] Therefore, the early identification of the populations in the first stages of a psychological crisis would allow for the efficient implementation of interventional strategies.[3] Clinicians would devise appropriate measures, including suggestions for professional psychological consultation and prioritizing the vulnerable patients for treatment. To the best of our knowledge, no study has compared the psychological status of individuals with OFP and the general population under the added psychosocial burden imposed by the COVID-19 pandemic. Therefore, this study aimed to compare the psychological distress, symptoms of depression and anxiety in the general population and individuals suffering from OFP during the COVID-19 pandemic. Moreover, the knowledge, perception, attitude, and concerns were compared between the groups.

Methods

Subjects

The study was conducted in Shiraz, Iran. Individuals aged 19–39 years were recruited in this study. The convenient consecutive sampling approach was used. Individuals with past or current neurologic or psychiatric illnesses or systemic diseases, or any oral and maxillofacial surgeries or orthodontic treatment in their treatment plan were excluded by asking the inviters not to invite them to participate in the study and ensure it with similar questions in the questionnaire. The respondents were divided into 2 groups as follows:

– group 1 – patients suffering from chronic pain in the orofacial region for more than the last 3 months, diagnosed by the clinicians through history, physical examinations and validated pain questionnaires (Brief Pain Inventory, Beck Depression Inventory, Hospital Anxiety and Depression Scale, McGill Pain Questionnaire, and Oral Impact on Daily Performance). The records and documents of the patients, from the date of the announcement of the beginning of the pandemic (March 11, 2020) to April 11, 2020, in 3 pain clinics and 3 dental emergency centers, were searched, and eligible subjects were invited to fill in the questionnaire. The same administrator was introduced to all the patients via a phone call by the clinic. The nature and purpose of the research were explained to the patients by the administrator through the WhatsApp messenger. The patients were allowed to ask the administrator questions via the messenger;

– group 2 – the general population with no diagnosis of OFP. The general population was invited to participate in the study through social media, including Instagram accounts, WhatsApp groups or Telegram channels, with more than 1,000 followers.

Ethical considerations

The protocol of this cross-sectional study was approved by the Ethics Committee of the Vice-Chancellor for Research at Shiraz University of Medical Sciences in Iran (No. IR.SUMS.DENTAL.REC.1399.122). The participants were told about the nature and purpose of the research by 1 administrator. The participants were allowed to ask the administrator questions through the WhatsApp messenger. They were reassured that all their personal data would be kept confidential. Informed consent was obtained from all the participants.

Questionnaire

The questionnaire consisted of 3 sections (Fig. 1).

The 1st section focused on individual background information and the person's primary source of information about COVID-19. Questions regarded the current orthodontic treatment, the current OFP and its treatment, and 6 questions related to the probability of catching coronavirus, job closure and quarantine. Questions about the exclusion criteria were also asked in this section.

Section 1——Basic information

1. Gender: 1) Male 2) Female
2. Age:
3. Education level:
1) Some high school education 2) High school graduate, associate degree
3) BA, MA 4) Doctorate and higher
4. Household size:
1) Alone 2) 2 people 3) 3-5 people 4) 6 people or more
5. Annual household income:
1) <240000000 Rials 2) 240000000-599999999 Rials
3) 600000000-1200000000 Rials 4) >1200000000 Rials
6. Marital status: 1) Single 2) Married 3) Divorced 4) Widowed
7. Residence: 1) City 2) Rural area
8. What is your occupation/major?
1) Non-medical related 2) Medical related
9. Do you have medical insurance? 1) Yes 2) No
10. Have you experienced job closure or layoffs during the COVID-19 outbreak? 1) Yes 2) No
11. During the COVID-19 outbreak, did you live alone?
1) Yes 2) No
12. Do you have fever, fatigue, coughs, dyspnea, and other common symptoms of the new coronavirus pneumonia? 1) Yes 2) No
13. Do you have a chronic disease? 1) Yes 2) No
14. Do you have a history of neurological or mental illness?
1) Yes 2) No

Section 1——Continued

15. During the COVID-19 outbreak, did you have any close contacts with individuals diagnosed with coronavirus?
1) Yes 2) No
16. Are you undertaking self-COVID-19 testing during the past two weeks? 1) Yes 2) No
17. Did you experience self-quarantine during the past 2 weeks?
1) Yes 2) No
18. What is your main source of obtaining information about COVID-19?
1) Radio 2) Television 3) Social media 4) Valid papers and websites 5) other
19. Do you experience orofacial pain, including severe dental pain, jaw joint pain or chronic headache during the COVID-19 outbreak?
1) Yes 2) No
20. Did you receive dental treatment in case of orofacial pain during the COVID-19 outbreak?
1) Yes 2) No
21. Are you currently under orthodontic treatment?
1) Yes 2) No

Section 2——Knowledge, perception, attitude, and concerns

Knowledge:

1. What is the COVID-19 transmission route?
1) Contact routes 2) Airborne transmission 3) Droplet transmission 4) All of the above
2. What is the main organ of COVID-19 involvement?
1) Brain 2) Heart 3) Lungs 4) Kidneys

Perception:

1. What do you think of the amount of your effort to take preventive measures?
Very little 1 2 3 4 5 Very much
2. In general, how well do you think you know about COVID-19?
Very little 1 2 3 4 5 Very much

Attitude:

1. In general, what do you think of the severity of the new coronavirus pneumonia?
Very little 1 2 3 4 5 Very much
2. What do you think is the possibility that you get infected with the new coronavirus?
Very little 1 2 3 4 5 Very much
3. What do you think is the possibility that the people around you get infected with the new coronavirus?
Very little 1 2 3 4 5 Very much
4. What do you think is the likelihood of surviving if infected with COVID-19
Very little 1 2 3 4 5 Very much

Concerns: What are your main concerns for the outbreak of the new coronavirus pneumonia? (Choose only one)
1) Isolation from the family and/or society
2) Impact on your work/study
3) Impact on daily life from measures like the lockdown of the city and community
4) Psychological barriers and distrust among people

Section 3——Evaluation of the psychological distress: *Please choose the option that suits you during the past four weeks.*

	None of the time	Small part of the time	Sometimes	Most of the time	All of the time
Did you feel tired for no good reason?					
Did you feel nervous?					
Did you feel so nervous that nothing could calm you down?					
Did you feel hopeless?					
Did you feel restless or fidgety?					
Did you feel so restless that you could not sit still?					
Did you feel depressed?					
Did you feel that everything was an effort?					
Did you feel so sad that nothing could cheer you up?					
Did you feel worthless?					

Fig. 1. Questionnaire used in the study, comprising 3 sections

The 2nd part consisted of 9 questions on knowledge (2 multiple-choice questions), perception (2 questions on a 5-point Likert scale; the higher the total score, the higher perception of the participant), attitude (4 questions on a 5-point Likert scale; the higher the total score, the more negative attitude of the individual), and the main concerns about the influence of the pandemic (a single-choice question and the participants could choose only 1 option) developed by the investigators.

The validity of the 2nd part of the questionnaire was assessed by submitting the questionnaire to 1 professor in each field – orthodontics, public health and internal medicine. The reliability was assessed by asking 20 subjects to complete it twice with a 2-week interval. Cronbach's α was used as a measure of reliability. All the values were ≥0.8.

The last part of the questionnaire was the Kessler Psychological Distress Scale (K10).[21] The reliable and valid Persian version of this scale was available at the beginning of the study.[22–24] There are 10 items (on a 5-point Likert scale) on this scale. The total score was regarded as a continuous variable in this study, with higher scores indicating increased psychological distress. The K10 score is a sensitive screen for diagnosing anxiety and depressive symptoms, with 4 items indicating anxiety (K-anxiety) and 6 items indicating symptoms of depression (K-depression).[25,26] A total score of 10–19 is considered normal, while 20–50 indicates mental distress (20–24: mild disorder; 25–29: moderate disorder; 30–50: severe disorder).[27]

Data collection

The questionnaires were developed at www.docs.google.com and filled in through an online survey. The questionnaires were available from April 25, 2020 to May 20, 2020; during that period, all routine elective medical and dental procedures were suspended except for emergencies.

The questionnaires with missing data or identical answers in all the K10 questions, or with extreme variations in answers regarding similar questions were excluded.

Statistical analysis

The data was imported to IBM SPSS Statistics for Windows, v. 21.0 (IBM Corp., Armonk, USA). The scores referring to the K10 questions were summed separately to form individual K10 scale, K10-anxiety and K10-depression scores for each respondent. The scores of knowledge, perception and attitude were summed separately to achieve the knowledge scores, the perception scores and the attitude scores, respectively. All the statistical analyses were performed with a two-tailed α significance level of 0.05, and 95% confidence interval (CI) for β and p-value was calculated.

All the background variables except for age were compared between the groups with the use of the χ^2 test. Age as well as the knowledge, perception and attitude scores were compared between the 2 groups with Student's *t* test. The primary concerns were compared between the groups by means of the χ^2 test.

Regardless of the group, the correlation of age and the knowledge, perception and attitude scores with the Kessler scores was determined using Pearson's correlation test. A comparison of the Kessler scores between various concern choices was evaluated using the one-way analysis of variance (ANOVA) and the post-hoc Šidák test. The Kessler scores were also compared between genders with Student's *t* test.

The one-way analysis of covariance (ANCOVA) was conducted to determine differences between the groups in the K10 scale, K-anxiety and K-depression scores, controlling for the possible confounding variables. The percentages of individuals with mental distress were also compared between the groups by means of the χ^2 test. Also, in group 2, one-way ANCOVA was used to compare the Kessler scores in the respondents who "sought pain management" and those who did not.

Table 1. Comparison of the background variables between the study groups

Background variables	Group 1 *n* = 112	Group 2 *n* = 397	*p*-value
Gender *n* (%)			
male	23 (20.54)	100 (25.19)	0.310
female	89 (79.46)	297 (78.81)	
Age [years] *M* ±*SD*	28.33 ±6.03	28.46 ±5.90	0.832
Education *n* (%)			
some high school education	3 (2.68)	10 (2.52)	0.225
high school graduate, associate degree	12 (10.71)	45 (11.34)	
bachelor's/master's degree	57 (50.89)	160 (40.30)	
doctorate and higher	40 (35.71)	182 (45.84)	
Household size *n* (%)			
alone	1 (0.89)	12 (3.02)	0.483
2 people	21 (18.75)	59 (14.86)	
3–5 people	78 (69.64)	280 (70.52)	
6 people or more	12 (10.71)	46 (11.59)	
Annual household income [rials] *n* (%)			
<240,000,000	11 (9.82)	43 (10.83)	0.016*
240,000,000–599,999,999	36 (32.14)	124 (31.23)	
600,000,000–1,200,000,000	44 (39.29)	104 (26.20)	
>1,200,000,000	21 (18.75)	126 (31.74)	
Marital status *n* (%)			
single	71 (63.39)	270 (68.01)	0.386
married	41 (36.61)	124 (31.23)	
divorced	0 (0)	3 (0.76)	
widowed	0 (0)	0 (0)	
Residence *n* (%)			
city	108 (96.43)	385 (96.98)	0.661
rural	4 (3.57)	12 (3.02)	
Occupation *n* (%)			
medical-related	61 (54.46)	251 (63.22)	0.093
non-medical-related	51 (45.54)	146 (36.78)	
Medical insurance *n* (%)	77 (68.75)	265 (66.75)	0.691
Experience of job closure/layoffs *n* (%)	76 (67.86)	275 (69.27)	0.775
Living alone during the pandemic *n* (%)	12 (10.71)	44 (11.08)	0.912
History of pneumonia-like symptoms *n* (%)	3 (2.68)	5 (1.26)	0.286
Close contact with individuals diagnosed with coronavirus *n* (%)	17 (15.18)	38 (9.57)	0.091
Undertaking self-COVID-19-testing during the past 2 weeks *n* (%)	5 (4.46)	20 (5.04)	0.804
Self-quarantine during the past 2 weeks *n* (%)	68 (60.71)	253 (63.73)	0.559
Main source of obtaining information about COVID-19 *n* (%)			
radio	0 (0)	2 (0.50)	0.691
television	27 (24.11)	93 (23.43)	
social media	59 (52.68)	212 (53.40)	
valid papers and websites	21 (18.75)	81 (20.40)	
other	5 (4.46)	9 (2.27)	

Group 1 – patients with orofacial pain (OFP); group 2 – the general population; *M* – mean; *SD* – standard deviation; COVID-19 – coronavirus disease 2019; * statistically significant. For all the background variables, the *p*-value was calculated with the χ^2 test except for age (Student's *t* test).

Results

The number of eligible patients in group 1 was 197. Temporomandibular disorders were present in 92 subjects (87 subjects with TMD of muscle origin, 5 subjects with TMD of joint origin). Headache was present in 84 subjects (53 subjects with headache of chronic tension type, 21 with chronic migraine and 10 with chronic daily headache). The remaining eligible 21 subjects had trigeminal neuralgia (15 subjects) and traumatic neuroma (6 subjects). Twenty-seven subjects with OFP did not fill the questionnaire (response rate: 86.3%). Totally, 635 respondents were recruited (group 1 or patients with OFP: $n = 170$; group 2 or the general population: $n = 465$). Respondents who met the exclusion criteria, or provided incomplete or mixed data were excluded (126 individuals). Finally, 509 eligible respondents (group 1: $n = 112$; and group 2: $n = 397$) underwent the statistical analysis.

Comparison of independent variables between the groups

Table 1 presents the demographic data and the background variables which were compared between the groups. The household income variable was statistically different between the study groups ($p = 0.016$).

The knowledge, perception and attitude scores of the respondents were not significantly different between the study groups ($p > 0.05$) (Table 2). The concerns of the respondents were not different between the study groups ($p > 0.05$). The most often reported main concern for both groups was "impact on work/studies" (Table 2).

Relationship between psychological indices and the respondents' age, knowledge, perception, attitude, and main concerns, regardless of the group

Age had a weak inverse correlation with the K10 scale ($r = –0.118$; $p = 0.003$), K-anxiety ($r = –0.114$; $p = 0.004$) and K-depression scores ($r = –0.116$; $p = 0.003$).

Attitude had a moderate positive correlation with the K10 scale ($r = +0.226$; $p = 0.0001$), K-anxiety ($r = +0.215$; $p = 0.0001$) and K-depression scores ($r = +0.224$; $p = 0.0001$). Perception had a weak negative correlation with the K10 scale ($r = –0.108$; $p = 0.005$), K-anxiety ($r = –0.103$; $p = 0.008$) and K-depression scores ($r = –0.106$; $p = 0.006$). Knowledge had a weak positive correlation with the K-anxiety score ($r = +0.077$; $p = 0.046$), while it was not statistically correlated with the K10 scale and K-depression scores ($p > 0.05$).

The K10 scale, K-anxiety and K-depression scores were not statistically different between the respondents with different concerns except for the respondents who chose "psychological barriers and distrust"; they exhibited significantly higher K10 scale ($p = 0.018$), K-anxiety ($p = 0.045$) and K-depression ($p = 0.014$) scores as compared to the respondents with the primary concern of "isolation from family/society".

Table 2. Comparison of knowledge, perception, attitude, and various concerns between the study groups

Variables	Group 1 *n* = 112	Group 2 *n* = 397	*p*-value
Knowledge *M* ±*SD*	7.71 ±1.24	7.59 ±1.43	0.411
Perception *M* ±*SD*	12.24 ±2.94	11.91 ±2.63	0.283
Attitude *M* ±*SD*	1.88 ±0.32	1.89 ±0.31	0.821
Concern *n* (%)			0.373
isolation from family/society	12 (10.71)	58 (14.61)	
impact on work/studies	52 (46.43)	151 (38.04)	
impact on daily life	33 (29.46)	122 (30.73)	
psychological barrier and distrust	15 (13.39)	66 (16.62)	

* statistically significant. For all the variables, the *p*-value was calculated with Student's *t* test except for concern (χ^2 test).

Comparison of psychological indices between genders and the groups, and within the groups

Regardless of the respondents' group, females exhibited significantly higher K10 scale, K-anxiety and K-depression scores than males ($p = 0.002$, $p = 0.002$ and $p = 0.003$, respectively).

A one-way ANCOVA was conducted to determine statistically significant differences between the groups in terms of the K10 scale, K-anxiety and K-depression scores, controlling for the household income. The K10 scale, K-anxiety and K-depression scores were significantly higher in group 1 than in group 2 ($p = 0.0001$; $F_{K10} = 18.31$; $F_{K\text{-anxiety}} = 16.06$; $F_{K\text{-depression}} = 18.29$) (Fig. 2).

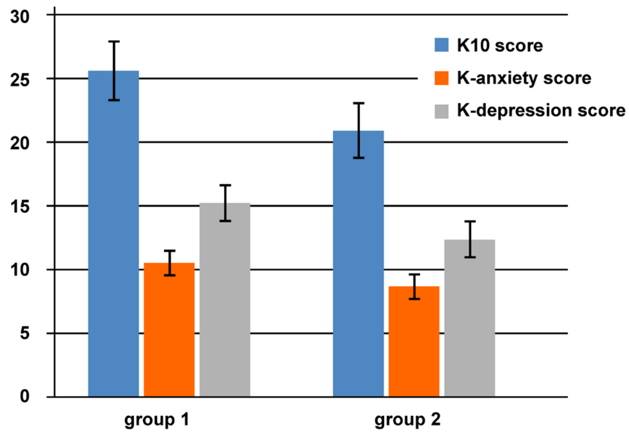

Fig. 2. Comparison of the adjusted psychological indices (*M* ±*SD*) between the study groups

K10 – Kessler Psychological Distress Scale; K-anxiety score – Kessler anxiety score; K-depression score – Kessler depression score.

Group 1 exhibited a significantly higher percentage of individuals with mental distress than group 2 (p = 0.047) (Fig. 3).

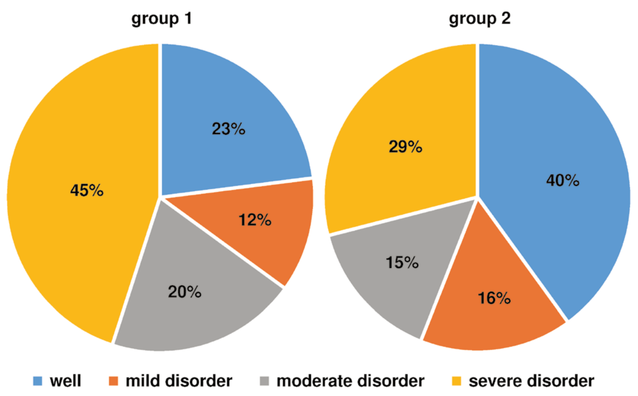

Fig. 3. Comparison of the frequency [%] of psychological distress between the study groups

In group 1, 10.71% (n = 12) of the respondents sought pain management; they did not differ from the respondents in their group who did not seek treatment in terms of the K10 scale and K-anxiety and K-depression scores ($p > 0.05$).

Discussion

The COVID-19 pandemic has imposed a substantial psychological burden on the world population, especially at the beginning of the outbreak, when individuals encountered unexpected quarantine, unemployment and uncertainty associated with the outbreak.[7] Pandemic-associated psychological factors might even exacerbate OFP.[19] The treatment outcome of OFP can also be influenced by depressive symptoms.[13] Therefore, the early identification of the populations in the first stages of a psychological crisis would allow for the efficient implementation of interventional strategies.[3] This study analyzed anxiety and depression in 509 respondents, with 397 normal population individuals and 112 patients with OFP, during the COVID-19 pandemic. The factors associated with COVID-19-related psychological distress which were determined in previous studies[28–30] were evaluated in our study. If they were different between the groups, they were adjusted not to affect the final comparison of the psychological status of the groups. Respondents with chronic illnesses and orthodontic treatment, or oral and maxillofacial surgeries were excluded, as these factors have been reported to affect the psychological condition.[31,32] In the present study, as the index of the psychological status, the Kessler Psychological Distress Scale was adopted[21]; it was previously used to assess the association between psychological distress and different types of pain, including musculoskeletal pain[33] and all types of chronic pain.[34]

Our socio-demographic data suggests that females experienced a more significant psychological impact of the outbreak, and had higher levels of anxiety and symptoms of depression. This finding corresponds to a previous extensive epidemiological study, which found that women were at higher risk of depression.[28] A study on Iranian medical students similarly revealed that anxiety was more prevalent in females than males; however, the study showed no significant difference between genders in depression.[35] This contrast can be attributed to the fact that medical students respond differently to the pandemic-related psychological burden as compared to the general population. As previously reported in another research,[30] older individuals exhibited less psychological distress in the present study, although the correlation was weak.

High levels of knowledge (score: 1.88–1.89 out of 2) and perception (score: 7.59–7.80 out of 10) in both groups represents success with regard to awareness measures and information programs. Higher knowledge of COVID-19 was associated with higher anxiety in the present study. It has been suggested that a higher level of information about COVID-19 received from various sources or the excessive use of media reporting on COVID-19 predict more anxiety among individuals,[36,37] which confirms our results. However, higher perception and a more positive attitude were found to be related to lower psychological distress. Previously, similarly to our findings, higher cognitive perception of the COVID-19 risk was reported to be related to a lower risk of depression for people in public health crises.[38] Another study revealed that regardless of the actual amount of knowledge individuals had, those perceiving themselves as more knowledgeable exhibited a stronger sense of control and experienced more happiness during the outbreak.[39]

Moreover, our findings are consistent with the positive correlation between attitude and psychological quality of life assessed in previous studies, in which the higher the coping attitude about the disease was, the higher the score in the psychological domain was obtained.[40,41] Keeping in mind the knowledge–attitude–behavior theory, there is a complex interaction between knowledge, perception and attitude.[42] Therefore, during the COVID-19 pandemic, all these variables should be addressed and improved concomitantly to enhance the related psychological status.

As for the main concerns about COVID-19, objective choices, like the impact on work/studies and daily life, were more important, while more subjective ones were relatively less important, such as the isolation from the family/society, psychological barriers and distrust among people. The main concern for individuals with OFP as well as for the general population was "impact on work/studies".

The respondents who chose "psychological barriers and distrust" as the primary concern exhibited significantly higher psychological distress than the respondents with the primary concern of "isolation from family/society".

This is somehow justified by the results of a study on adults indicating that some cognitive strategies, such as rumination, catastrophizing and self-blame, are linked to poorer psychological well-being.[43] In contrast, other strategies, like positive refocusing, putting into perspective and acceptance, show few significant associations with poorer psychological health.[43]

The main results of the present study indicated that the K10 scale, K-anxiety and K-depression scores were noticeably higher in participants with OFP than in the other group. The frequency of mental distress was higher in the OFP group.

This higher level of anxiety and depressive symptoms in individuals with OFP suggests that people with a history of pain experience would be possibly the main targets of psychiatric assessment and care. In a recent systematic review there was consistent evidence that chronic pain was associated with PTSD,[44] which confirms our results. Previously, OFP, especially chronic pain, was associated with lower oral health-related quality of life, higher suicidal ideation, depression, anxiety, PTSD, and psychological distress signs.[13,18,44] The comparison of the limited number of population-based studies is difficult due to different psychological indices and methods used.[45–48] None of the previous studies assessed the psychological status of patients with OFP in comparison with the general population after the psychological effect of an accident. However, our results are similar to those reported by Kindler et al., concerning psychological distress, and specifically the role of anxiety and depression related to pain; the authors found a moderate-to-strong relationship between the symptoms of depression or anxiety and the signs of TMD.[46] Also, Natu et al. stated that the severity of TMD had some bearing on the quality of life, emotional states and sleep quality.[47] Dindo et al. suggested that psychological inflexibility was related to the expression of the symptoms of anxiety and depression in adults with migraine.[48] The results of a recent study by Simoen et al. before the pandemic indicated that patients with pain attributed to TMD had higher depression and anxiety scores in comparison with the general population.[49] Although different psychological indices were applied (Patient Health Questionnaire-9 and Generalized Anxiety Disorder-7), their findings were similar to ours during the pandemic.[49] A recent study showed that the aggravation of the psychoemotional status caused by the COVID-19 pandemic could result in the exacerbation of bruxism and TMD symptoms, and even lead to increased OFP.[20]

When people with chronic pain are denied assessment and treatment, their condition can worsen significantly, decreasing health-related quality of life, increasing pain and exacerbating depression.[50,51] Efforts are suggested to prevent and control pain – particularly chronic pain – during the COVID-19 pandemic. Moreover, addressing emergencies associated with pain, avoiding medication shortages due to panic-buying and avoiding the inaccessibility of the remaining healthcare options during movement restrictions are recommended, and pain management providers face the challenge of delivering face-to-face services through different modes.[52]

Most patients with OFP did not seek dental/medical pain management during the pandemic. A study suggested that precautionary measures, such as avoiding sharing utensils, hand hygiene and wearing masks to prevent the spread of COVID-19, could have had protective psychological effects during the early stages of the pandemic.[28] Therefore, it might be concluded that the process of diagnosis and treatment of patients can be resumed with appropriate preventive measures so that they are not afraid of referring for pain management.

Overall, social media were the primary health information channels in both groups during the COVID-19 pandemic. Similarly to our findings, other studies reported that participants usually obtained information about the novel coronavirus through social media.[53,54] It should be noted that this large platform should be used for raising awareness and training in high-risk groups in the coronavirus pandemic, and the content of health information provided during the pandemic should be based on evidence to avoid adverse psychological reactions.[28] On the other hand, this familiarity of the population with social media can be taken as an advantage. Telehealth, the sourcing of treatment modalities by means of digital and telecommunication technologies, can be provided by health professionals to exchange information necessary for self-care as well as the diagnosis, treatment and prevention of pathologies and injuries, including OFP or psychological distress.[55]

Varying degrees of the outbreak severity, national economy, government preparedness, the availability of medical supplies/facilities, a proper dissemination of COVID-related information, and cultural differences have led to regional differences in the general public's psychological health during the pandemic.[7] The COVID-19 outbreak began in Iran on February 19, 2020, and promptly spread all over the country. On April 21, 2020, of 330,137 tested patients, 80,868 were infected with COVID-19 (55,987 recovered, 3,513 became critically ill and 5,031 died). Like everywhere in the world, the formal announcement of the outbreak resulted in public panic and anxiety. Fake news and misinformation further increased public anxiety. The Headquarter for Coronavirus Combat and Prevention implemented strategies, such as stopping mass gatherings, the closure of educational institutes, national coordination with volunteer, civilian and military forces, the national screening program, and social distancing. These measures potently alleviated some of the public fear. However, at the same time, they could affect the economy. People were urged to strongly avoid familial gatherings and trips during the New Year holidays. Stricter measures, especially travel bans, were introduced on March 26, 2020. The measures desirably lead to the flattening of the epidemic curve.[56] In the subsequent months, the government gained control of the virus and began relaxing lockdown measures. At the beginning of June 2020, the media reported a worrying sharp increase in the number of COVID-19 cases that mirrored March peak levels: 3,574 new infections in 24 h as of June 3.[57]

This is the first report on the psychological distress of individuals with OFP as compared to the general population during the COVID-19 pandemic, to the best of our knowledge. The main strengths of the present investigation are the comparison of the severity of this psychological distress and a considerable number of participants.

Like other questionnaire-based research, the accuracy of our study results depended on the participants' accuracy in answering the questionnaire. Our respondents were mostly females and were of the young adult group. Due to ethical requirements concerning anonymity and confidentiality, we were not allowed to collect contact details and personal information from the respondents. As a result, we could not conduct a prospective study that would provide concrete evidence to support the need for a focused public health initiative. Given the limited resources available and the time-sensitivity of the COVID-19 outbreak, we adopted the non-randomized, convenient sampling strategy. Due to the cross-sectional design, the associations in the study cannot be perceived as causal. Lastly, this study had some limitations in interpreting the results, since COVID-19 is a novel coronavirus disease and limited research is available for comparison.

Future studies on a comparable number of males and females within various age groups are suggested. Furthermore, it would be ideal to conduct a prospective study on the same group of participants after some time, especially when all routine dental and medical procedures, including elective ones, are allowed. Future studies with a similar examination approach in a population-based group and a patient group are recommended to draw more logical conclusions.

Conclusions

In summary, our findings suggested that during the rapid rise of the COVID-19 outbreak, the psychological status was associated with knowledge, perception, attitude, and concerns about COVID-19. Females suffered more from psychological distress than males. As compared to the general population, psychological distress, and symptoms of depression and anxiety were noticeably higher in individuals with OFP, who suffered from moderate psychological distress.

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Original papers

Serum levels of vitamin D in patients with recurrent aphthous stomatitis

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Abstract

Background. Recurrent aphthous stomatitis (RAS) is one of the most common recurrent ulcerations in the oral mucosa, the etiology of which has not been elucidated; the immune system dysfunction may play an important role in the pathogenesis of RAS. The anti-inflammatory and regulatory role of vitamin D in the functioning of the immune system is well-documented.

Objectives. This study aimed to evaluate and compare the serum levels of vitamin D between patients with RAS and healthy controls.

Material and methods. In this case–control study, 43 patients with minor RAS and 43 healthy controls were included. Two groups were matched in terms of age and sex. Blood samples were obtained from all participants. The serum levels of vitamin D were measured with the use of the enzyme-linked immunosorbent assay (ELISA) in patient and control groups. The data was analyzed using the SPSS for Windows software, v. 17.0, with the independent samples t test and the Mann–Whitney test. A p-value of <0.05 was considered statistically significant.

Results. The mean serum level of vitamin D in the control group was significantly higher than that in the case group (22.59 $\pm$16.06 ng/mL vs 13.19 $\pm$8.19 ng/mL, respectively; $p = 0.002$).

Conclusions. The serum levels of vitamin D are lower in patients with RAS in comparison with healthy controls.

Key words: vitamin D, immune system, recurrent aphthous stomatitis

Introduction

Recurrent aphthous stomatitis (RAS) is one of the most common recurrent ulcerations in the oral mucosa. It mostly occurs in societies with a high socioeconomic status and affects 5–25% of the population.[1] The lesions are self-limiting. Pain and oral dysfunction are the most important complications of chronic inflammatory lesions.[2]

Recurrent aphthous stomatitis is classified into 3 clinical forms: minor; major; and herpetiform ulcerations, with the minor form being the most common.[2] Multiple factors, including genetic and hematologic disorders, immunological deficiency, stress, and trauma, are known as predisposing for RAS.[3,4] Many researchers have evaluated the role of the immune system dysfunction in aphthous stomatitis. According to their studies, at the early stages of RAS, $CD4^+$ T lymphocytes are predominantly involved, and at the ulcerative stages – $CD8^+$ T lymphocytes.[5] An increase in Th1 pro-inflammatory cytokines and a decrease in Th2 anti-inflammatory cytokines in the pathogenesis of RAS are confirmed.[6,7] Recent studies have shown the role of vitamin D in skeletal disorders, cardiovascular diseases, cancers, and autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematous and type 1 diabetes mellitus.[8–10] The anti-inflammatory and regulatory role of vitamin D in the functioning of the immune system is well-documented.[9] Vitamin D receptor (VDR) is found in most cells of the immune system, including antigen-presenting cells (APCs). Serum 25(OH)D is considered the most accurate marker for vitamin D status. It is well known that the measurement of 25(OH)D levels is the best indicator of the vitamin D status in humans.[10,11]

1,25(OH)-vitamin D3 is the metabolically active form of vitamin D which inhibits the maturation of dendritic cells, and shifts the balance of the differentiation pathway from Th1 and Th17 into Th2 and regulatory T cells, respectively. The inflammatory response is increased by Th1 and decreased by Th2. Therefore, vitamin D modulates the immune system through reestablishing the balance between the immune system components.[11–13]

However, the role of vitamin D in the pathogenesis of RAS is not clear, and contradictory results have been reported. Öztekin and Öztekin showed a decreased serum level of vitamin D in patients with RAS,[10] whereas Krawiecka et al. reported no significant difference between patients with RAS and healthy individuals in terms of serum vitamin D levels.[7]

Objectives

Therefore, the current study aimed to compare the serum levels of vitamin D in RAS patients and healthy individuals.

Material and methods

The participants in this case–control study were referred to the Department of Oral and Maxillofacial Medicine of the School of Dentistry at Babol University of Medical Sciences in Iran (Ethics committee code: IR.MUBABOL.HRI.REC.1397.204).

The sample size for each group (n = 43) was calculated with a 95% confidence level, a study power of 80%, and by considering a 5-unit difference in the serum levels of vitamin D between the 2 groups.

Individuals with RAS, aged 15–40 years, were included in the case group. An aphthous ulcer was diagnosed based on the clinical manifestation (1 or 2 minor RAS ulcers, <1 cm in diameter, located in the buccal or labial mucosa) and at least 3 periods of minor RAS per year. The control group consisted of healthy individuals, and was matched in terms of age and sex with the case group.

The exclusion criteria for both case and control groups were as follows: any systemic disease; malnutrition; smoking; oral lesions other than RAS; the consumption of vitamin D and calcium supplements in the last 6 months; pregnancy; and lactating.

The participants were informed of the study design and provided written informed consent. Their medical history and clinical examination were recorded in the checklist.

Blood samples were collected by 1 laboratory technician from all participants in the laboratory of the Shahid Yahya Nejad hospital in Babol, Iran, during spring and summer (April–September). A total of 5 cc of blood was drawn from each patient. Then, serum was separated from whole blood; it was centrifuged at 2,500 × g for 10 min and was kept at −80°C until the analysis was performed.

The measurement of vitamin D level was performed by means of the enzyme-linked immunosorbent assay (ELISA), using a laboratory kit (Cat. No. EUROIMMUN, EQ. 6411-9601; PerkinElmer, Lübeck, Germany) based on the present protocol of the kit.

All test steps were conducted using Elisys Uno, a fully automated ELISA analyzer (HUMAN, Wiesbaden, Germany).

According to serum vitamin D level, each participant was classified as 'normal' (30–50 ng/mL), 'insufficient' (20–30 ng/mL) or 'deficient' (<20 ng/mL).

Finally, the data was analyzed with the independent samples t test and the Mann–Whitney test, using the SPSS for Windows software, v. 17.0 (SPSS, Inc., Chicago, USA), with the significance level set at 0.05.

Results

In this study, out of 86 individuals, 43 individuals (11 men and 32 women) were in the case group and 43 individuals (8 men and 35 women) were in the control group, and the 2 groups were matched in terms of sex (p = 0.600).

The mean age was 32.56 ±7.93 years in the case group and 33.74 ±7.07 years in the control group, and there was no statistically significant difference between the groups in this respect (p = 0.460).

The mean serum level of vitamin D in the control group was 22.59 ±16.06 ng/mL and in the RAS patients, it was 13.89 ±8.19 ng/mL. Vitamin D level in the control group was significantly higher than in the case group (p = 0.002) (Fig. 1).

Vitamin D categories in both groups are shown in Table 1. There was a statistically significant difference between the groups according to vitamin D categories (p = 0.032)

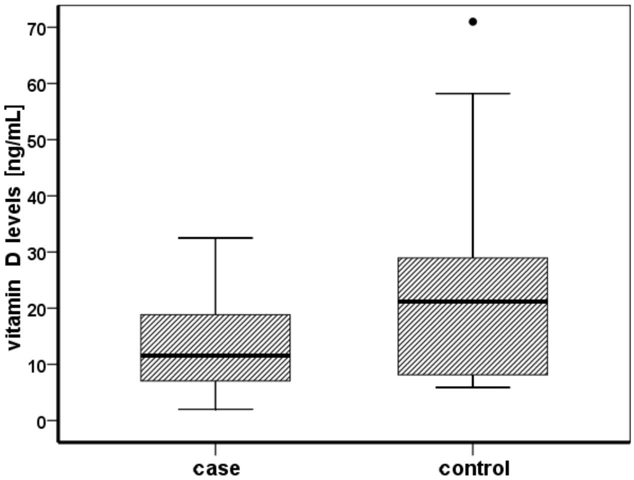

Fig. 1. Comparison of 25(OH)D concentrations between recurrent aphthous stomatitis (RAS) patients and the control group (p = 0.002)
Data presented as mean (M) ± standard deviation (SD).

Table 1. Vitamin D categories in the study groups

25(OH)D category	Case group	Control group	p-value
Vitamin D-deficient	32 (74.4)	21 (48.8)	
Vitamin D-sufficient	9 (20.9)	12 (27.9)	0.032*
Vitamin D-normal	2 (4.7)	10 (23.3)	

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

Discussion

In the present study, the serum levels of vitamin D in patients with minor RAS were lower than those in healthy individuals.

Few studies have evaluated the possible relationship between RAS and serum vitamin D level. In a study by Öztekin and Öztekin,[10] similar to Bahramian et al.'s research,[14] patients with RAS had vitamin D deficiency. According to Nalbantoğlu and Nalbantoğlu, the serum levels of vitamin D in children with RAS, aged 3–12 years, were decreased.[15] The results of the above-mentioned studies are in line with our results, thus supporting the thesis that there is a relationship between vitamin D level and RAS. On the contrary, Krawiecka et al. reported different results, implying no significant correlation between the serum level of vitamin D and RAS.[7]

The level of 25(OH)D was measured in all studies assessing serum vitamin D.

It is well documented that 25(OH)D is the most appropriate marker to assess vitamin D status in humans.[11] According to the confounding effect of age and sex on the serum level of vitamin D, matching for these 2 variables in both study groups decreases the bias; thus, in the current study, the effects of confounders were controlled.

The contradictory results obtained by Krawiecka et al. could be due to the lack of matching for sex.[7]

Lower serum levels of vitamin D in RAS patients as compared to the control group in Krawiecka et al.'s study might have been statistically significant if gender had been matched.[7]

In this study, the mean serum level of 25(OH)D was 13.89 ±8.19 ng/mL in the RAS group and 22.59 ±16.06 ng/mL in the control group, which was reported lower by Öztekin and Öztekin,[10] and higher by Bahramian et al.[14] and Nalbantoğlu and Nalbantoğlu.[15]

Although there are various methods to assess the serum levels of vitamin D, including chemiluminescent immunoassay (CLIA), high-performance liquid chromatography (HPLC) and radioimmunoassay (RIA), there is no standard established. This variety of measuring methods may result in reporting different serum levels of vitamin D.[16,17]

In addition, differences in the measured serum level of vitamin D between the studies might be due to gender, age, geographical region, seasonal changes, diet, ethnicity, and culture.[16,17]

Vitamin D deficiency may be related to a higher melanin content in the skin and the use of extensive skin coverage. Hence in Middle East countries, including Iran, serum 25(OH)D levels are usually low.[7,18,19]

In the United States, 47% of African American infants and 56% of Caucasian infants have vitamin D deficiency, while in Iran, Turkey and India, over 90% of infants have vitamin D deficiency.[19]

Since RAS etiology is not clear and several factors are thought to contribute, there is no standard and definite treatment for it. Therapeutic options include local analgesics, corticosteroids and immune modulators, which are non-specific and administered based on the patient's symptoms.[10,20]

Some studies have investigated the therapeutic role of various vitamins in treating aphthous stomatitis.

Lalla et al. showed that vitamin supplements (A, B1, B2, B3, B5, B6, B9, B12, C, D, and E) had no effect on the number and duration of aphthous lesions.[20] However, Pederson et al. reported multivitamin to be effective in treating RAS, even when the serum levels of vitamin D were appropriate.[21]

A number of researchers suggested the role of innate and acquired immunity in the pathogenesis of RAS, which leads to the activation of neutrophils and the complement system, an increase in the number of NK cells and B lymphocytes, and an imbalanced $CD4^+/CD8^+$ ratio.[4,22,23]

There are shreds of evidence supporting the regulatory role of vitamin D in the cellular and humoral immunity function. Most cells of the immune system, including APCs (e.g., macrophages, dendritic cells and T cells) express the hormone VDR.[11,24]

The biological effects of vitamin D, including the regulation of innate and acquired immunity as well as its influence on the cytokine profile, imply the role of this hormone in RAS progression.[11,24]

A decrease in the serum level of vitamin D is reported in other autoimmune disorders, such as Behçet's disease, the periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome, inflammatory bowel disease (IBD), rheumatoid arthritis, and autoimmune disorders of thyroid.[7,8,10]

Limitations

In the current study, the severity of aphthous lesions was not assessed. Therefore, further research with larger sample sizes is needed to investigate the intensity of aphthous lesions.

In addition, clinical trial studies to evaluate the potential therapeutic and protective role of vitamin D in RAS are warranted.

Conclusions

With our findings, the serum levels of vitamin D in patients with RAS were significantly lower than those in healthy individuals.

Given the regulatory effects of vitamin D on the immune system, and having considered the results of the current study, it can be concluded that low serum levels of vitamin D might be a probable etiologic factor for RAS, especially in genetically susceptible patients.

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Cytotoxicity evaluation of calcium hypochlorite and other commonly used root canal irrigants against human gingival fibroblast cells: An in vitro evaluation

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Abstract

Background. The conventional endodontic therapy primarily focuses on biomechanical preparation, which is achieved by the application of various intracanal irrigants and intracanal medicaments. One of the most commonly used intracanal irrigants – sodium hypochlorite (NaOCl) – has already been proven to have an antimicrobial effect as well as the ability to dissolve tissues in the areas where files cannot reach. One of the recently used irrigants having a promising effect is calcium hypochlorite ($Ca(OCl)_2$), which has been shown to be relatively more stable than NaOCl and has much more chlorine ions.

Objectives. The aim of this study was to assess the individual cytotoxicity of various root canal irrigants and the combined cytotoxicity of NaOCl and $Ca(OCl)_2$ with ethylenediaminetetraacetic acid (EDTA) against human gingival fibroblast (hGF) cells.

Material and methods. The evaluation of the individual cytotoxicity was carried out with regard to the following root canal irrigants: NaOCl; $Ca(OCl)_2$; and chlorhexidine (CHX). The evaluation of the combined cytotoxicity regarded NaOCl/EDTA and $Ca(OCl)_2$/EDTA. The concentrations used were 0.025%, 0.050%, 0.10%, and 0.20%. The cytotoxicity against hGF cells was examined within a timeframe of 6 h and 24 h with the use of the sulforhodamine B (SRB) assay.

Results. It was observed that $Ca(OCl)_2$ had a mean absorbance rate of 0.315 ±0.02, 0.294 ±0.03, 0.265 ±0.03, and 0.240 ±0.02 at 0.025%, 0.050%, 0.10%, and 0.20%, respectively. In combination with EDTA, the mean absorbance rate was 70.12 ±2.9, 67.42 ±4.3, 64.35 ±3.6, and 61.58 ±4.1 at 0.025%, 0.050%, 0.10%, and 0.20%, respectively. The cytotoxic effect of the root canal irrigants on hGF cells was observed to be statistically significant ($p < 0.05$).

Conclusions. Calcium hypochlorite is less cytotoxic than NaOCl, and when used in combination with EDTA, it was shown to have its cytotoxic effect on hGF cells reduced to a great extent.

Key words: chlorhexidine, EDTA, endodontics, root canal irrigants, sodium hypochlorite

Introduction

The typical aim in the endodontic therapy is to perform the optimal disinfection of the root canal system, after which a suitable filling material is placed in this disinfected space, preventing any further ingress of microorganisms leading to further complications.[1] It is well known that microorganisms are a critical factor in the development of pulp and periapical diseases, and have an influence on the prognosis of the endodontic therapy in the long run.[2,3] Currently, along with mechanical instrumentation, various adjuvants, such as intracanal irrigants and intracanal medicaments are used, which helps to significantly reduce the amount of microorganisms. Due to the complexity of the root canal anatomy, intracanal irrigants are of great importance in the endodontic procedure, since they have the ability to get into the sites where mechanical instrumentation fails to reach.[4]

Various intracanal irrigants have been used in the conventional endodontic therapy in the past decades in order to help the clinician to completely eradicate microbes, necrotic tissues and debris from the root canal system, the most common being chlorhexidine (CHX) and sodium hypochlorite (NaOCl), which have been shown to have an antimicrobial effect, the ability to inactivate endotoxins as well as the ability to remove some part of the smear layer and necrotic tissue remnants.[5] Sodium hypochlorite, one of the most common intracanal irrigants, also shows antimicrobial activity and is able to dissolve pulpal tissue remnants in spaces such as fins and ramifications, where instrumentation cannot reach.[6] The capability of NaOCl to dissolve pulpal tissue results from its alkaline nature as well as from the release of $HOCl^-$ and OCl^- ions.[7]

Sodium hypochlorite is known to show certain drawbacks, such as the lack of substantivity, the ability to dissolve only the organic portions of the smear layer and a cytotoxic effect when extruded into the periapical region. One of the ways to overcome these drawbacks is to use it in combination with ethylenediaminetetraacetic acid (EDTA), which reduces the pH of NaOCl in a time-dependent manner as well as the amount of the available free chlorine, making NaOCl more stable and preventing the weakening of the dentinal matrix.[8] One of the recently introduced intracanal irrigants of a promising potential is calcium hypochlorite ($Ca(OCl)_2$), which has shown an antimicrobial effect and at the same time a much higher chlorine release in comparison with NaOCl.[9] These irrigants have been reported to affect various cells present in the periapical complex. Although numerous assessments of their cytotoxicity against multiple cell lines have been performed, there is no data available in the dental literature on the cytotoxic effect of these irrigants on human gingival fibroblast (hGF) cells. The current study aimed to evaluate the individual cytotoxic activity of various root canal irrigants, and the combined cytotoxicity of NaOCl and $Ca(OCl)_2$ with EDTA against hGF cells.

Material and methods

This in vitro study had received prior approval of the institutional review board (No. SRB/SDC/ENDO-1805/20/04).

Preparation of the cell line

This in vitro study was conducted under aseptic conditions in a research laboratory. The hGF cell lines were received from the National Centre for Cell Science, Pune, India. The hGF cells were seeded in the α-minimal essential medium (α-MEM) containing 10% fetal bovine serum (FBS), 100 IU/mL penicillin, 2.5 μg/mL streptomycin, 2.5 μg/mL amphotericin B, and 50 μg/mL ascorbic acid, which was replaced twice a week, and incubated in 5% CO_2 at 37°C for humidification.

Preparation of calcium hypochlorite and other irrigants

The $CaOH_2$ powder of 65% purity (Merck, Darmstadt, Germany) was weighed on a precision balance and mixed with distilled and sterilized water. When the solutions were completely dissolved, they were filtered twice to remove debris, and then stored in bottles. Other chemicals used in the study were as follows: NaOCl (Molychem, Mumbai, India); EDTA (Thermo Fisher Scientific, Waltham, USA); and CHX (Sigma-Aldrich, St. Louis, USA).

Experimental groups

The individual cytotoxicity evaluation was done in 4 groups:

- group A: 10% NaOCl diluted to 0.025%, 0.050%, 0.10%, and 0.20%;
- group B: 10% $Ca(OCl)_2$ diluted to 0.025%, 0.050%, 0.10%, and 0.20%;
- group C: 2% CHX diluted to 0.025%, 0.050%, 0.10%, and 0.20%;
- group D: the growth medium used as a control group.

For the combined cytotoxicity evaluation, 2 groups were analyzed:

- group A: 10% NaOCl diluted to 0.025%, 0.050%, 0.10%, and 0.20%, mixed with EDTA;
- group B: 10% $Ca(OCl)_2$ diluted to 0.025%, 0.050%, 0.10%, and 0.20%, mixed with EDTA.

Cytotoxicity test with the sulforhodamine B assay

The cytotoxicity test was performed using a modification of the method developed by Vichai and Kirtikara.[10] The assessment was carried out within a timeframe of 6 h and 24 h, with each concentration being tested in triplicate. The microtiter plates were incubated with Dulbecco's Modified Eagle's Medium (DMEM)

(Life Technologies, Grand Island, USA) supplemented with 10% FBS, 100 µL/well of the culture medium, for 24 h at 37°C with 5% CO_2. After 24 h of incubation, cell adhesion and growth were observed. The cellular concentration used was 1.4 × 104 cells/mL. The medium was then discarded, and the treatment group samples, controls and DMEM without FBS were added to each well. After 24 h of incubation, the cells were fixed with trichloroacetic acid (TCA) (100 µL/well) and placed under refrigeration for 1 h at 4°C. Trichloroacetic acid was then removed, and the plates were washed in low-flow water 3 times and dried. After that, the plates were stained for 20 min at room temperature with sulforhodamine B (SRB) (Sigma-Aldrich) 0.4% (50 µL/well). The plates were washed with 1% acetic acid solution and dried at room temperature. The unbound dye was removed after washing; the dye bound to the protein was solubilized in the basic medium to determine the optical density in a plate reader set at 570 nm. The colorimetric evaluation gives an estimate of total protein mass. The cellular concentration used was 1.4 × 104 cells/ml. The percentage of living cells for each concentration of the tested substances was assessed with the following equation (Equation 1):

$$\text{percentage of cells survived} = \frac{(\text{AbsT} - \text{AbsC})}{(\text{AbsC})} \times 100 \quad [\%] \; (1)$$

where:
AbsT – average absorbance of the tested substances;
AbsC – average absorbance of the control.

Statistical analysis

The statistical analysis was carried out using IBM SPSS for Windows, v. 21.0 (IBM Corp., Armonk, USA) The one-way analysis of variance (ANOVA) was applied to assess differences in absorbance at 570 nm at different time intervals between the treatment groups, post-hoc Tukey's test was used for the intergroup comparison of absorbance between the test groups; the independent *t* test was done to assess cell viability at different time points between the groups. The level of significance was set at $p < 0.05$.

Results

The experiments were carried out in triplicate. The results of the assessment of cytotoxicity against hGF cells are shown in a dose-dependent and time-dependent manner, with a total timeframe of 6 h and 24 h. They are presented as mean (*M*) ± standard deviation (*SD*).

Table 1 shows the absorbance values for different root canal irrigants at different concentrations at 6 h and 24 h. Table 2 presents the percentage of cell viability for different root canal irrigants at different concentrations at 6 h and 24 h. The findings of the study suggest that CHX, NaOCl and $Ca(OCl)_2$ had an effect on hGF cells (p = 0.001). Figure 1 shows the dose-dependent effect of various root canal irrigants on hGF cells at 6 h. The intergroup analysis of the rate of absorbance at the 0.025% concentration showed statistically significant differences in various combinations: NaOCl vs $Ca(OCl)_2$ (p = 0.002); NaOCl vs negative control (NC) (p = 0.001); $Ca(OCl)_2$ vs CHX (p = 0.013); and CHX vs NC (p = 0.001).

Table 1. Absorbance values for various root canal irrigants at different concentrations at 6 h and 24 h

Irrigant	Absorbance at 570 nm at 6 h				Absorbance at 570 nm at 24 h			
	0.025%	0.050%	0.10%	0.20%	0.025%	0.050%	0.10%	0.20%
NaOCl	0.125 ±0.04	0.129 ±0.01	0.105 ±0.01	0.052 ±0.01	0.286 ±0.02	0.258 ±0.01	0.222 ±0.05	0.202 ±0.04
$Ca(OCl)_2$	0.315 ±0.02	0.294 ±0.03	0.265 ±0.03	0.240 ±0.02	0.498 ±0.06	0.468 ±0.02	0.424 ±0.03	0.390 ±0.03
CHX	0.175 ±0.03	0.148 ±0.02	0.112 ±0.1	0.098 ±0.02	0.312 ±0.09	0.238 ±0.04	0.221 ±0.06	0.192 ±0.05
NC	0.392 ±0.04	0.388 ±0.03	0.381 ±0.04	0.396 ±0.05	0.512 ±0.03	0.507 ±0.03	0.512 ±0.04	0.512 ±0.05

NaOCl – sodium hypochlorite; $Ca(OCl)_2$ – calcium hypochlorite; CHX – chlorhexidine; NC – negative control.
Data presented as mean (*M*) ± standard deviation (*SD*).

Table 2. Cell viability for various root canal irrigants at different concentrations at 6 h and 24 h

Irrigant	Percentage of viable cells [%] at 6 h				Percentage of viable cells [%] at 24 h			
	0.025%	0.050%	0.10%	0.20%	0.025%	0.050%	0.10%	0.20%
NaOCl	52.78 ±2.6	41.89 ±1.6	32.60 ±2.5	20.80 ±1.4	51.78 ±4.3	48.56 ±2.6	39.89 ±2.8	23.82 ±2.1
$Ca(OCl)_2$	69.89 ±1.8	60.23 ±3.4	53.99 ±1.6	50.16 ±2.4	56.64 ±5.6	50.11 ±0.6	42.71 ±3.1	28.15 ±1.9
CHX	79.35 ±3.2	71.54 ±2.4	68.21 ±3.8	70.25 ±3.9	70.25 ±6.4	63.58 ±3.1	59.96 ±4.6	53.89 ±4.3
NC	100.00 ±9.6	100.00 ±8.2	100.00 ±7.5	100.00 ±5.7	100.00 ±8.1	100.00 ±2.6	100.00 ±8.3	100.00 ±9.1

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

The comparison of absorbance at the 0.050% concentration showed statistically significant differences in the following cases: NaOCl vs $Ca(OCl)_2$ ($p = 0.001$); NaOCl vs NC ($p = 0.001$); $Ca(OCl)_2$ vs CHX ($p = 0.001$); $Ca(OCl)_2$ vs NC ($p = 0.001$); and CHX vs NC ($p = 0.001$). At the 0.10% concentration, differences in absorbance were observed to be statistically significant for NaOCl vs $Ca(OCl)_2$ ($p = 0.001$), NaOCl vs NC ($p = 0.001$), $Ca(OCl)_2$ vs CHX ($p = 0.001$), $Ca(OCl)_2$ vs NC ($p = 0.002$), and CHX vs NC ($p = 0.001$). At the 0.20% concentration, the comparison of absorbance showed statistically significant differences for NaOCl vs $Ca(OCl)_2$ ($p = 0.001$), NaOCl vs NC ($p = 0.001$), $Ca(OCl)_2$ vs CHX ($p = 0.005$), $Ca(OCl)_2$ vs NC ($p = 0.006$), and CHX vs NC ($p = 0.001$).

Figure 2 shows the dose-dependent effect of various root canal irrigants on hGF cells at 24 h. The comparison of the absorbance of various irrigants at 0.025% showed statistically significant differences for the following: NaOCl vs $Ca(OCl)_2$ ($p = 0.004$); NaOCl vs NC ($p = 0.003$); $Ca(OCl)_2$ vs CHX ($p = 0.004$), $Ca(OCl)_2$ vs NC ($p = 0.003$), $Ca(OCl)_2$ vs NC ($p = 0.008$), and CHX vs NC ($p = 0.005$). At the 0.050% concentration, the comparison of absorbance showed statistically significant differences for NaOCl vs $Ca(OCl)_2$ ($p = 0.002$), NaOCl vs NC ($p = 0.001$), $Ca(OCl)_2$ vs CHX ($p = 0.001$), and CHX vs NC ($p = 0.001$). At the 0.10% concentration, the intergroup comparison of absorbance showed statistically significant differences for NaOCl vs $Ca(OCl)_2$ ($p = 0.001$), NaOCl vs NC ($p = 0.001$), $Ca(OCl)_2$ vs CHX ($p = 0.001$), and CHX vs NC ($p = 0.001$). In the case of the 0.20% concentration, the intergroup absorbance rate was observed to be statistically significantly different for NaOCl vs $Ca(OCl)_2$ ($p = 0.008$), NaOCl vs NC ($p = 0.001$), $Ca(OCl)_2$ vs CHX ($p = 0.008$), $Ca(OCl)_2$ vs NC ($p = 0.006$), and CHX vs NC ($p = 0.001$).

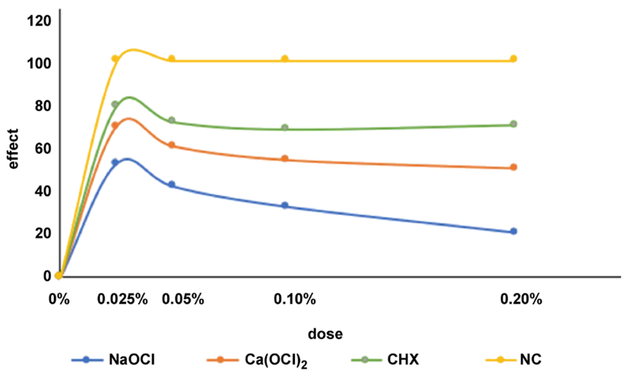

Fig. 1. Dose–effect plot of sodium hypochlorite (NaOCl), calcium hypochlorite ($Ca(OCl)_2$) and chlorhexidine (CHX) at 6 h against human gingival fibroblast (hGF) cells (sulforhodamine B (SRB) assay)

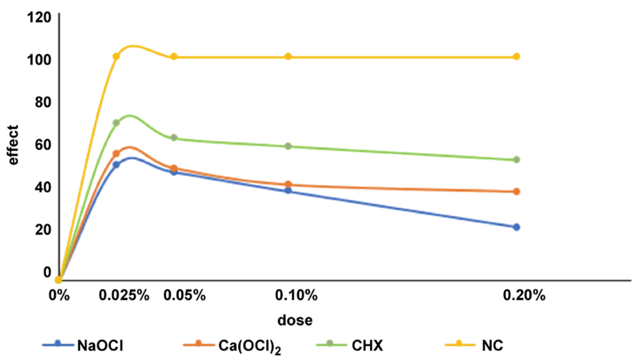

Fig. 2. Dose–effect plot of sodium hypochlorite (NaOCl), calcium hypochlorite ($Ca(OCl)_2$) and chlorhexidine (CHX) at 24 h against human gingival fibroblast (hGF) cells (sulforhodamine B (SRB) assay)

Table 3 shows the percentage of cell viability for hGF cells post reaction with NaOCl/EDTA and $Ca(OCl)_2$/EDTA at different concentrations at 6 h and 24 h. Table 4 presents the final concentrations of NaOCl and $Ca(OCl)_2$ after serial dilution with EDTA at 6 h and 24 h. Figure 3 depicts the combined cytotoxicity against hGF cells at 6 h of the combinations of NaOCl/EDTA and $Ca(OCl)_2$/EDTA. The comparison of cell viability done at 6 h showed statistically significant difference between NaOCl/EDTA and $Ca(OCl)_2$/EDTA at 0.025% ($p = 0.001$), 0.050% ($p = 0.001$), 0.10% ($p = 0.001$), and 0.20% ($p = 0.001$). Figure 4 shows the combined cytotoxicity against hGF cells at 24 h for the combinations of NaOCl/EDTA and $Ca(OCl)_2$/EDTA. The comparison of cell viability done at 24 h showed statistically significant difference between NaOCl/EDTA and $Ca(OCl)_2$/EDTA at 0.025% ($p = 0.019$), 0.050% ($p = 0.007$), 0.10% ($p = 0.005$), and 0.20% ($p = 0.029$).

Table 4. Final concentration after serial dilution with ethylenediaminetetraacetic acid (EDTA) at 6 h and 24 h

Time [h]	NaOCl/EDTA	$Ca(OCl)_2$/EDTA
6	0.025/0.1	0.005/0.2
	0.050/0.2	0.012/0.3
	0.100/0.3	0.025/0.4
	0.200/0.4	0.037/0.5
24	0.037/0.01	0.015/0.05
	0.015/0.02	0.037/0.04
	0.023/0.03	0.050/0.03
	0.041/0.04	0.075/0.02

Table 3. Cell viability at 6 h and 24 h in combination with ethylenediaminetetraacetic acid (EDTA) (sulforhodamine B (SRB) assay)

Combination of irrigant/EDTA	Percentage of viable cells [%] at 6 h				Percentage of viable cells [%] at 24 h			
	0.025%	0.050%	0.10%	0.20%	0.025%	0.050%	0.10%	0.20%
NaOCl/EDTA	51.24 ±3.1	50.43 ±2.3	39.43 ±3.1	30.56 ±1.9	50.12 ±2.1	41.89 ±3.5	34.16 ±2.4	28.49 ±2.9
$Ca(OCl)_2$/EDTA	70.86 ±4.5	66.89 ±5.8	64.32 ±2.8	63.47 ±2.8	70.12 ±2.9	67.42 ±4.3	64.35 ±3.6	61.58 ±4.1

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

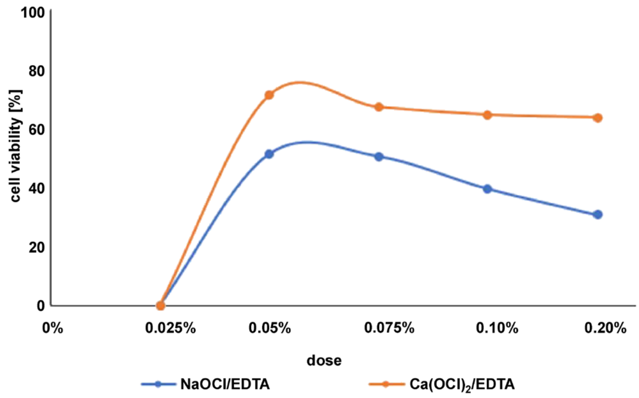

Fig. 3. Dose–cell viability plot of sodium hypochlorite (NaOCl) with ethylenediaminetetraacetic acid (EDTA) and calcium hypochlorite ($Ca(OCl)_2$) with EDTA at 6 h against human gingival fibroblast (hGF) cells (sulforhodamine B (SRB) assay)

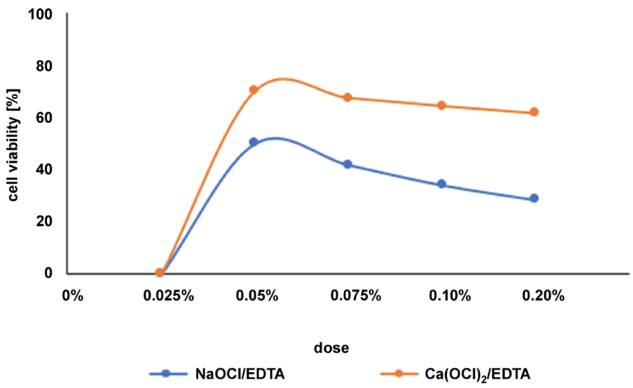

Fig. 4. Dose–cell viability plot plot of sodium hypochlorite (NaOCl) with ethylenediaminetetraacetic acid (EDTA) and calcium hypochlorite ($Ca(OCl)_2$) with EDTA at 24 h against human gingival fibroblast (hGF) cells (sulforhodamine B (SRB) assay)

Discussion

Root canal irrigants have a pivotal role in the success of the endodontic therapy. It is well known that they participate in removing necrotic tissue and debris from fins and ramifications, where mechanical instrumentation cannot reach. Still, there is a risk that during the endodontic procedure, intracanal irrigants will be extruded periapically.[11] This can cause damage to cells and also impair wound healing.[12] Another potential effect of the extrusion of irrigants is that they may impair the survival of stem cells, which are required for successful tissue regeneration.[13]

Human gingival fibroblast cells are reported to have a much higher reparative potential than human periodontal ligament fibroblasts (hPDLF).[14] Though hPDLF cells could simulate periodontal ligament fibroblasts better, they are known to produce a higher amount of collagen than hGF cells, and could potentially influence the results of cytotoxicity for the materials tested.[15] Apart from having great regenerative properties as compared to other fibroblasts, hGF cells also have the multilineage capability to differentiate into different types of cells, such as osteoblasts and periodontal ligament cells, which can be potentially used for periodontal tissue engineering.[16] The presence of lipopolysaccharides in endodontic pathogens has an immunosuppressive effect on the human pulp, leading to pulpal pain and periapical inflammation.[17] Unlike other cells present in the periodontium, hGF cells do not express lipopolysaccharide tolerance and have been shown to release inflammatory cytokines for up to 7 days, which makes them the least susceptibile to microbial action.[18]

As compared to other standard cell lines, hGF cells are diploid host cells, continuous in nature, with the specialized properties retained; they are more likely to demonstrate the cytotoxic effect exerted by dental materials on cells than other commercially available cell lines, which are aneuploid in nature, showing a heteroploid chromosome pattern and responding differently to different materials.[19] The use of commercially available standardized cell lines has an advantage that they are easily accessed, but can demonstrate greater toxicity levels and show an altered effect of the use of dental materials as compared to specific lineage cell lines, which are more desirable, as they bridge the gap between in vitro and in vivo biocompatibility.[20]

Various studies have already been done in the field of endodontics, using hGF cells. Barnhart et al. assessed the cytotoxicity of various intracanal irrigants with the CyQUANT® assay and found potassium iodide and calcium hydroxide to be the least cytotoxic against hGF cells.[21] Lee et al. also evaluated the cytotoxicity of various dental adhesives against hGF cells and found that all of the tested adhesives had a cytotoxic effect on hGF cells.[22]

In the present study, the SRB assay was used for the cytotoxicity assessment, as it has been shown to have a higher sensitivity; the cell viability count is not affected by any compounds released from the specimen, is independent of any cellular metabolic action, which makes the assay much easier to conduct in comparison with other assay methods.[23]

Calcium hypochlorite is another intracanal irrigant introduced in endodontics which has been shown to have a good antimicrobial activity.[24–26] Though it is very similar to NaOCl in terms of chemical action, the presence of more chlorine (Cl^-) in $Ca(OCl)_2$ results in more pronounced antimicrobial action and a greater tissue dissolving capability as compared to NaOCl.[27] The current study showed the least cytotoxic effect in the case of CHX. It is known that CHX has a sustained antimicrobial activity as well as low toxicity, but its major disadvantage is its inability to dissolve organic pulp tissues.[28]

The present study showed that $Ca(OCl)_2$ maintained the viability of cells when tested within a timeframe of 6 h and 24 h. In contrast, NaOCl was proven to decrease the levels of viable hGF cells after 24 h even further. In an in vitro study, Coaguila-Llerena et al. found that differences in cytotoxicity against L929 and hPDLF cells between $Ca(OCl)_2$ and 2% CHX were statistically insignificant; also, the cellular proliferation capacity was maintained when $Ca(OCl)_2$ at a concentration of 2.5% was used.[29] In another study, Ferraz Blattes et al. conducted an in vitro cytotoxicity assessment against 3T3 fibroblasts and an analysis of the inflammatory reaction in rats; the authors reported that $Ca(OCl)_2$ showed lower levels of inflammation, better healing response as well as lower cytotoxicity as compared to NaOCl.[30]

The combined use of NaOCl with EDTA is the recommended protocol to be followed according to the American Association of Endodontists (AAE) guidelines and was the basis for evaluating the combined cytotoxic effect in our study. It is known that NaOCl/EDTA acts on the organic/inorganic portion of the smear layer present in dentin and shows a synergistic effect by chelating Ca^{2+} ions, causing collagen deproteinization, which results in a superficial change in dentin, preventing further microbial colonization.[31] The present study also showed that cytotoxicity was reduced to a greater extent with the addition of EDTA. Our results are consistent with those obtained by Vouzara et al., who proved that the application of the combination of NaOCl and EDTA reduced the cytotoxic effect against MRC5 cells.[32] The results obtained in our study could be due to an antagonizing effect of EDTA on NaOCl, which plays a critical role in reducing its pH in a time-dependent manner as well as decreasing exponentially the amount of the released chlorine gas.[33] Due to the chemically similar nature, the same action could be justified in the case of $Ca(OCl)_2$ as well.

Limitations

One of the limitations of the present study is not using an in vivo model to assess the cytotoxic reaction, since an in vitro model is an ideally conditioned environment and may not necessarily transmit the achieved results in vivo. Another limitation is probably the use of lower concentrations of irrigants, which were diluted further to different levels for assessment standardization and may not necessarily show the same result when used in their clinically recommended concentrations.

Conclusions

Based on the present study, it can be concluded that $Ca(OCl)_2$ showed less cytotoxicity in a time-dependent and dose-dependent manner than NaOCl, but was more cytotoxic against hGF cells in comparison with chlorhexidine gluconate. The combined use of $Ca(OCl)_2$ and NaOCl with EDTA showed a more significant reduction of cytotoxicity against hGF cells. In contrast, the sustainability of hGF cells after 24 h was much greater in the case of using $Ca(OCl)_2$ as compared to NaOCl.

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Original papers

High glucose promotes the aging of human dental pulp cells through Wnt/beta-catenin signaling

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

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Abstract

Background. Diabetes is one of the most common metabolic diseases that disrupt the functioning of different body organs, including oral tissue. Some diabetic complications remain even after the control of the hyperglycemic condition. The adverse effect of hyperglycemia on the pulp structure and function has been shown previously.

Objectives. The purpose of this study was to investigate the effect of the hyperglycemic state on the aging of pulp cells and evaluate the role of Wnt signaling as the underlying mechanism of this process.

Material and methods. The isolated pulp cells were cultured in the Minimum Essential Medium (MEM)-alpha for 7, 14 and 21 days under the influence of glucose at concentrations of 5 mM, 20 mM and 30 mM. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate cell viability, the beta-galactosidase test was applied to assess cellular senescence and gene expression was measured with quantitative real-time polymerase chain reaction (qRT-PCR).

Results. The results of this study showed that cell proliferation decreased following exposure to 20 and 30 mM glucose, which was accompanied by an increased number of senescent cells and an increased p21 expression. There was a significant increase in beta-catenin and Wnt1 expression in response to high glucose. Treatment with beta-catenin inducer enhanced cellular aging in the hyperglycemic state, while beta-catenin inhibitor decreased the senescence response.

Conclusions. The present study further confirmed the effect of the high-glucose condition on pulp cell aging and suggests a role for beta-catenin in the induction of cellular aging. Targeting the key regulators of cellular aging in pulp tissue might lead to the development of new therapies for the prevention and treatment of endodontic complications in diabetic patients.

Key words: Wnt signaling, beta-catenin, pulp stem cells, diabetes, senescence

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Introduction

Diabetes is among the most common metabolic diseases in the world, being responsible for millions of deaths per year. Diabetes is not only recognized as a disease, but also as a series of diseases that may affect the body's systems and many organs. The global prevalence of diabetes in 2010 was 8.3%, representing 387 million patients; the number is estimated to reach 552 million by 2030.[1,2] Impaired insulin secretion and function are typical features of the diabetic state, which is characterized by high blood glucose. Diabetes-induced changes in the metabolism of carbohydrates and lipids cause extensive modifications in the function and structure of the vascular and nervous systems, which consequently affect various organs, such as the heart, kidney, eyes, etc.

A large number of studies have shown the influence of high glucose on oral tissue.[3,4] People with diabetes are at higher risk of periodontal disease than healthy people. Indeed, diabetes has been identified as a major risk factor for periodontitis and gingivitis.[5] Other oral manifestations and complications of diabetes include salivary dysfunction, taste disturbance, increased prevalence of oral mucosa lesions, and poor wound healing capacity.[4] There are also profound changes in the alveolar bone function, accompanied by reduced osteogenic capacity and bone remodeling, which could lead to tooth loss. Studies on the adverse effects of diabetes on pulp tissue are limited. Based on histopathological observations, diabetic pulp undergoes significant changes, including increasing the basement membrane thickness, decreasing the lumen diameter and obliterative endarteritis, contributing to poor vasculature and a higher risk of pulp necrosis.[6] Also, calcifications in diabetic pulp are frequently observed, resembling age-associated changes in old individuals.[7] At the cellular level, hyperglycemia may enhance the expression of inflammatory factors and structural proteins, such as collagen and osteopontin,[8,9] which is accompanied by an increase in the expression of oxidative stress-related enzymes.[10] Mesenchymal stem cells (MSCs) residing in human pulp exhibit a high capacity of proliferation and odontoblastic differentiation, which was found to be reduced in diabetic rats, thus affecting the regeneration of dentin and the formation of the dentin bridge.[11] Still, the underlying mechanism of hyperglycemia-induced cellular and molecular changes in pulp tissue has not been fully elucidated. There is evidence showing the causative effect of hyperglycemia in the induction of cellular aging in different cell types as well as diabetic models.[12–14]

Understating the detailed molecular mechanism of diabetic-induced changes in the cell function and targeting the key regulatory factors can be of great importance in the development of new therapeutic approaches in endodontic treatment for patients suffering from diabetes. In the present study, the effect of high glucose on the induction of the senescence response in cultured pulp cells was investigated. Also, the potential role of Wnt signaling in high glucose-induced senescence was evaluated.

Material and methods

Material

Glucose was purchased from Merck (CAS 77938-63-7; Darmstadt, Germany). The inhibitor of Wnt/beta-catenin pathway PNU-74654 (CAS 113906-27-7) was from Santa Cruz Biotechnology (Santa Cruz, USA), and both 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) were from Roche Diagnostics (Mannheim, Germany). Lithium chloride (LiCl; CAS 7447-41-8), magnesium chloride ($MgCl_2$), potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium hexacyanoferrate (II) trihydrate ($K_4[Fe(CN)_6{\cdot}3H_2O]$), and dimethyl sulfoxide (DMSO) were purchased from Merck. Fetal bovine serum (FBS) and phosphate-buffered saline (PBS) were from Biowest (Nuaillé, France). The sources of all other materials used in the experiments were indicated in the text.

Primary cell culture, stem cell characterization and cell treatment

Pulp cells were isolated from dental pulp tissue. For this aim, the teeth extracted from 3 healthy patients attending the Dental Policlinic of Tehran University of Medical Sciences in Iran were transferred immediately to the laboratory in Minimum Essential Medium (MEM)-alpha (Biowest) with 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, pH 7.4. Informed written consent was obtained from the subjects for conducting the study. The tissues were digested in the collagenase-dispase enzyme solution (Invitrogen, Waltham, USA) and the primary cells were isolated according to the previously reported method.[15] The isolated cells were cultured in MEM-alpha supplemented with 10% FBS under the humidified condition at 37°C with 5% CO_2. The pulp cells were characterized by the expression of their surface cluster of differentiation (CD) markers and their potential to differentiate into different cell lineages according to the previously described techniques.[16]

To study the effect of glucose on pulp cells, a stock solution of 100 mg/mL glucose was prepared in MEM-alpha. The solution was filtered and the appropriate volume was added to each well containing the culture medium to give the total concentrations of 20 mM and 30 mM glucose. The pulp cells were treated with the medium containing 20 mM or 30 mM glucose for 7, 14 and 21 days. The control groups were treated with 5 mM glucose. The medium was refreshed every 3 days. The cells were exposed to LiCl (6 mM) or PNU-74654 (10 µM) on the same day of glucose treatment.

MTT assay

The cells were seeded in 96-well tissue culture plates at a density of 4,000 cells/well. After overnight rest time, the cells were treated with different concentrations of glucose in MEM-alpha supplemented with FBS. Cell proliferation was measured with the MTT assay, which is based on the cleavage of the tetrazolium salt (MTT) by metabolically active cells to form the purple formazan crystal dye. After removing the cell culture medium from each well, 100 μL of the MTT solution (50 mg/10 mL PBS) was added and the plate was incubated at 37°C for 3–4 h. Following the formation of formazan crystals, the MTT solution was substituted with 50 μL of DMSO to dissolve the crystals while shaking for 20 min. The absorbance was measured at wavelengths of 570 nm and 650 nm by means of a multi-well spectrophotometer (BioTek Instruments, Inc., Winooski, USA).

Beta-galactosidase assay

Beta-galactosidase cytochemical staining was used to evaluate cellular senescence. The beta-galactosidase enzyme is expressed in the lysosomes of all cells at pH 4, which is the optimum pH of the enzyme activity. The senescent cells with high beta-galactosidase activity are distinguished under high pH 6. For this experiment, the cultured cells in 24-well plates were washed with PBS and fixed with paraformaldehyde 2% in PBS for 5 min, which was followed by washing with PBS twice. The senescence-associated (SA)-beta-galactosidase staining solution was prepared in PBS containing $MgCl_2$ (2 mM), $K_4[Fe(CN)_6{\cdot}3H_20]$ (2.12 mg/mL), $K_3[Fe(CN)_6]$ (1.64 mg/mL), and X-Gal (1 mg/mL) at pH 6. The 500 μL of the solution was added to each well and the plate was incubated at 37°C. After 24 h, the staining solution was removed and the cells were rinsed twice in PBS. Finally, 500 μL of the SYBR® green solution (1:10,000) (Sigma-Aldrich, St. Louis, USA) was added to each well. The blue X-Gal-stained cells were counted under light microscopy (Leica Camera, Wetzlar, Germany). The number of blue cells was recorded in at least 10 different microscopic fields and the percentages were calculated from the total number of fluorescent nuclei that were detected in the same field under fluorescence microscopy.[17]

Real-time polymerase chain reaction and gene expression assessment

Total RNA was extracted from the control and treated samples with the use of TRI Reagent® (Sigma-Aldrich), with standard procedures based on phenol-chloroform extraction and ethanol precipitation. The extracted RNA was quality-controlled for purity and integrity with agarose gel electrophoresis and the measurement of the 260/280 nm ratio of absorbance. Next, 1 μg of RNA was treated with 0.1 U of RNase-free DNase I (Roche Applied Science, Mannheim, Germany) and converted to cDNA, using BioFact™ RT-Kit (BIOFACT, Daejeon, South Korea) according to the manufacturer's protocol. Subsequently, 2 μL of cDNA was used as a template for quantitative real-time polymerase chain reaction (qRT-PCR) with 1 μL of primers, 10 μL of BioFact™ 2X Real-Time PCR Master Mix and 1 μL of random hexamer, and 6 μL of sterile, diethyl pyrocarbonate (DEPC)-treated water. The conditions for qRT-PCR include: 15 min at 95°C for denaturation; 20 s at 95°C; 20 s at 60°C; and 30 s at 72°C.

The sequence of primers was as follows:
p21 F 5´-GGCACCCTAGTTCTACCTCA-3´,
R 5´-CTCCTTGTTCCGCTGCTAAT-3´;
Wnt-1 F 5´-GTTCCATCGAATCCTGCACG-3´,
R 5´-CTGCCTCGTTGTTGTGAAGG-3´;
beta-cat F 5´-TCCCTGAACTGACAAAACTGCT-3´,
R 5´-CACCATCTGAGGAGAACGCAT-3´; and
GAPDH F 5´-CACATGGCCTCCAAGGAGTAA-3´,
R 5´-TGAGGGTCTCTCTCTTCCTCTTG-3´.

The PCR products were visualized on 1% agarose gel and melting curves were evaluated for single peaks. The relative fold change was calculated relative to the control group after normalization to the internal control gene, glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). The formula $2^{-\Delta\Delta Ct}$ was applied as the standard method for determining gene expression changes.

Statistical analysis

The significance of the obtained data was determined using two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test with the help of the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA). All the experiments were performed at least twice, each in triplicate on separate dates. The level of significance was set at 0.05.

Results

Characterization of pulp mesenchymal stem cells

The extracted pulp MSCs successfully adhered to the plastic cell culture plates and were positive for MSC surface markers CD73, CD90 and CD105, as assessed by flow cytometry and the immunofluorescence analysis (Fig. 1A,1B). Also, the cells showed the lack of the expression of hematopoietic markers CD 45 and CD 34. The MSCs could differentiate into different cell types, including the osteogenic, adipogenic and chondrogenic lineages (Fig. 1C).

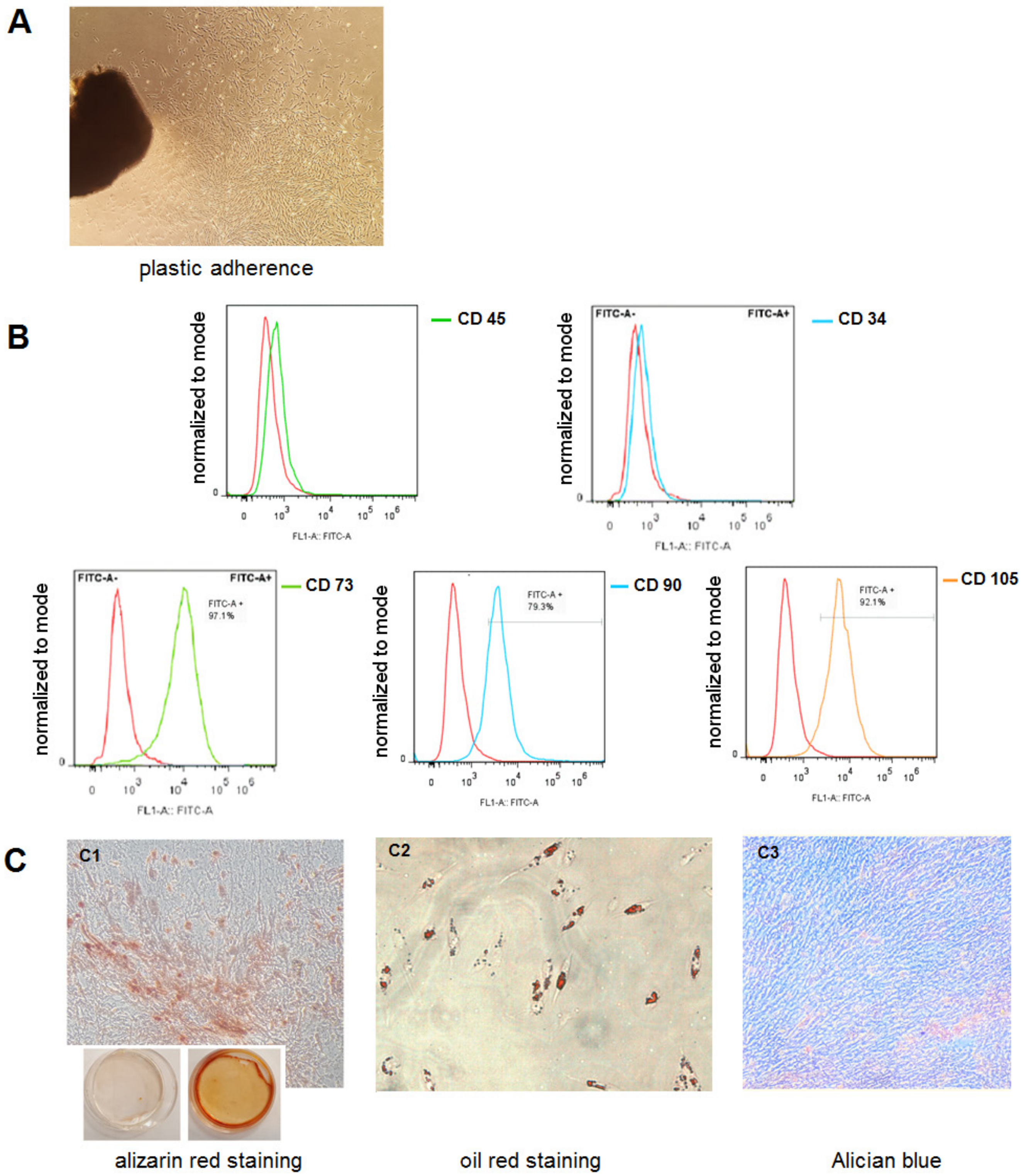

Fig. 1. Characterization of pulp mesenchymal stem cells (MSCs)

A – plastic adherence; B – MSC surface markers; C – differentiation of MSCs into osteoblasts (alizarin red staining), adipocytes (oil red staining) and chondrocytes (Alician blue staining).

High glucose reduced the proliferation of pulp cells

The proliferation rate of the isolated pulp cells was examined at a high concentration of glucose during long-term exposure by means of the MTT assay. The results showed that while the number of cells in the control group (5 mM glucose) increased continuously, exposure to 20 mM and 30 mM glucose significantly inhibited cells proliferation on days 14 and 21 (Fig. 2A).

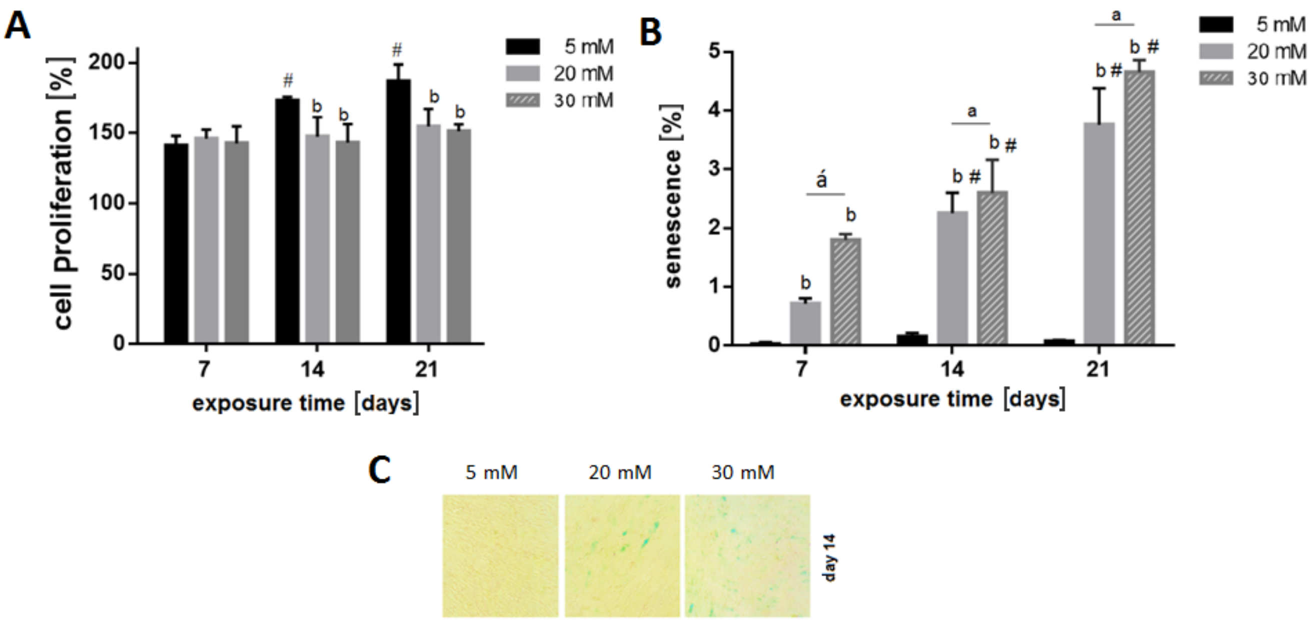

Fig. 2. Cell proliferation at different concentrations of glucose on days 7, 14 and 21 (A), the corresponding diagram of the percentage of the senescent cells increased at high glucose concentrations (B) and senescence-associated (SA) beta-galactosidase staining (the senescent cells with beta-galactosidase activity with blue color developed in their cytoplasm) (C)

[a] significantly different from group 20 mM on a specific day ($p < 0.05$); [á] significantly different from group 20 mM on a specific day ($p < 0.001$); [b] significantly different from control on a specific day ($p < 0.001$); [#] significantly different from control on day 7 ($p < 0.001$).
For A and B, data presented as mean (*M*) ± standard deviation (*SD*).

High glucose induced cellular senescence in pulp cells

To evaluate the presence of the senescent cells among the pulp cells treated with glucose, the beta-galactosidase assay was performed. The results showed that exposure to high concentrations of glucose increased SA beta-galactosidase activity in a dose-dependent manner (Fig. 2B,2C). The number of aging cells increased with the passing time and an increasing glucose concentration. To further confirm the senescence response, the expression of the p21 marker associated with cell cycle arrest was assessed. High glucose resulted in an enhanced expression of p21 after 7-, 14-, and 21-day exposure (Fig. 3).

Increased beta-catenin and Wnt1 expression at high glucose

There was a significant increase in the expression of beta-catenin at 30 mM glucose as compared to control 5 mM glucose on day 7 ($p < 0.001$). Exposure to 20 mM and 30 mM glucose enhanced Wnt1 expression in the cultured pulp cells (Fig. 4).

High glucose-induced senescence response is mediated by beta-catenin

To evaluate the role of Wnt signaling in the glucose-induced senescence response, the cells were treated with beta-catenin inhibitor – PNU-74654 – and beta-catenin inducer – LiCl. The results showed that LiCl aggravated cellular senescence in the presence of high concentrations of glucose (20 mM and 30 mM). Also, an increased percentage of senescent cells alleviated in the presence of PNU-74654 (10 μM) at high glucose concentrations (Fig. 5A). Similarly, LiCl induced p21 expression, while exposure to PNU-74654 reduced the p21 level under hyperglycemic conditions, as measured by qRT-PCR (Fig. 5B).

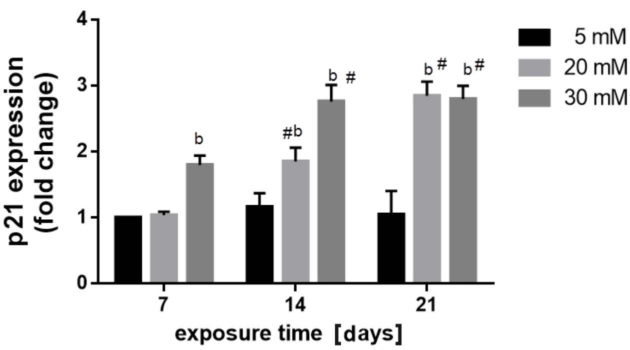

Fig. 3. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of gene expression of p21 in the cells exposed to different concentrations of glucose on days 7, 14 and 21

[b] significantly different from control on a specific day ($p < 0.001$);
[#] significantly different from control on day 7 ($p < 0.001$).
Data presented as *M* ±*SD*.

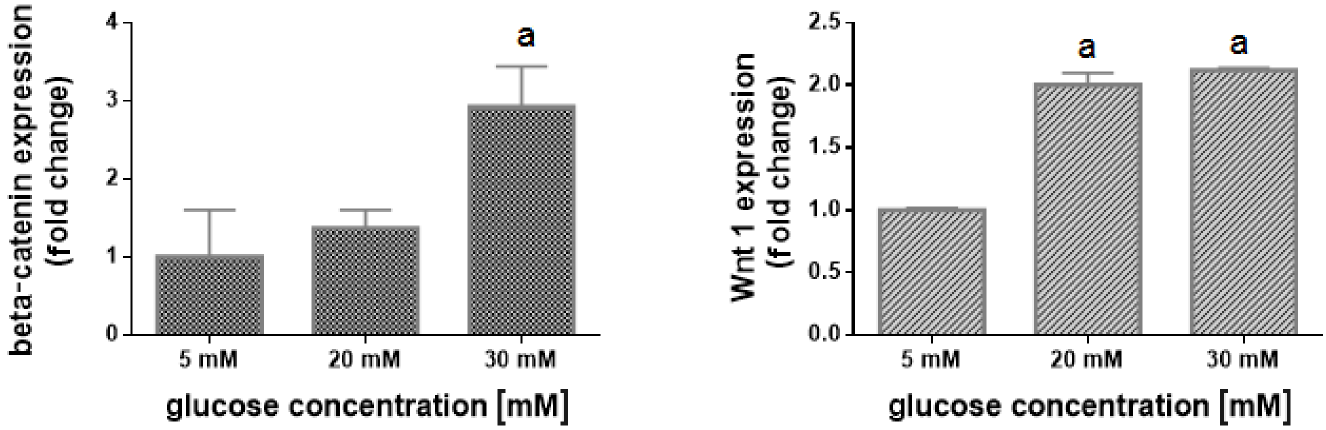

Fig. 4. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of gene expression of beta-catenin and Wnt-1 in the cells exposed to different concentrations of glucose

[a] significantly different from control ($p < 0.001$). The experiment was performed 3 times, each in triplicate.
Data presented as *M ±SD*.

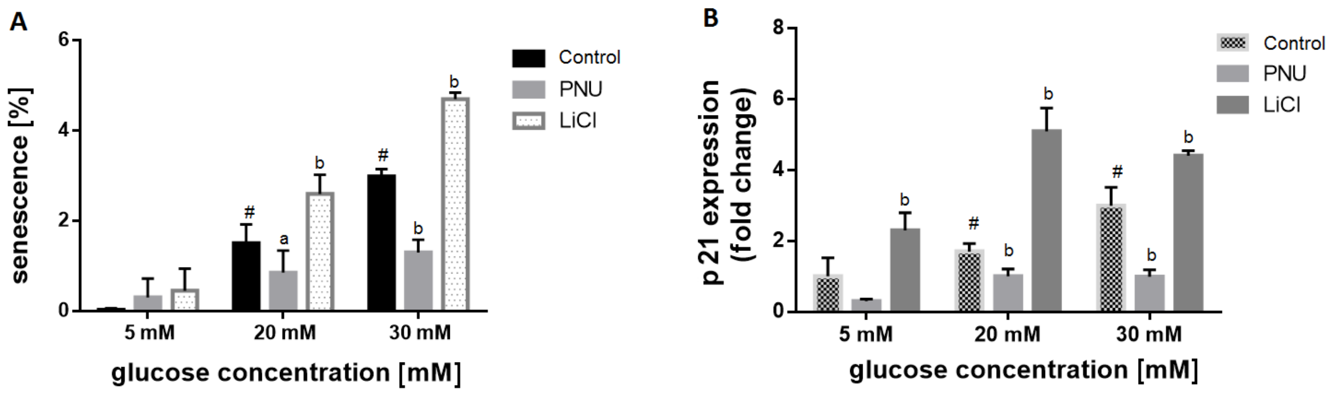

Fig. 5. Beta-galactosidase staining analysis and quantitative real-time polymerase chain reaction (qRT-PCR) analysis of p21 expression after exposing the cells to beta-catenin inhibitor (PNU-74654) and beta-catenin inducer (LiCl) at different glucose concentrations

A – percentage of the senescent cells after exposure to beta-catenin inducer or inhibitor at different glucose concentrations; B – expression of p21 in the cells after exposure to beta-catenin inducer or inhibitor at different glucose concentrations;
[a] significantly different from control on a specific day ($p < 0.05$); [b] significantly different from control on a specific day ($p < 0.001$);
[#] significantly different from control on day 7 ($p < 0.001$).
Data presented as *M ±SD*.

Discussion

Senescence or cellular aging is a process that irreversibly ceases the proliferation of normal cells in response to external environmental factors or internal stimuli, such as telomere length shortening and oncogene induction. While maintaining their metabolic activity, the senescent cells undergo phenotypic changes in their structure and secretory profile. In this study, we attempted to investigate the influence of high glucose on cells isolated from pulp tissue in an in vitro model resembling the hyperglycemic condition in diabetic patients.

Considering that the fasting blood glucose of diabetic patients rises above 10 mM, in the present study – in order to simulate the diabetic state – the concentrations of 20 mM and 30 mM were used, in accordance with the concentrations applied in previous studies.[18–20] The optimum concentration of 5 mM in the cell culture medium was selected as the control group. Our results showed that the culture medium enriched with glucose could pose an inhibitory effect on the proliferation rate after 2 and 3 weeks of exposure, indicating the chronic influence of the hyperglycemic medium.

The beta-galactosidase staining method is regarded as the key test for the evaluation of cellular aging based on the level of lysosomal beta-galactosidase activity at pH 6. While the optimum activity of the enzyme is at pH 4, only the senescent cells with strong enzyme activity are detectable at pH 6.[21] Our results showed that the number of beta-galactosidase-positive cells increased at the 20 mM and 30 mM glucose concentrations as compared to the control group. Along with beta-galactosidase activity, the expression of cyclin-dependent kinase (CDK) inhibitor p21 – the marker of cellular senescence – was increased. The data further confirmed the induction of senescence and cell cycle arrest during long-time exposure to the hyperglycemic condition.

Human pulp tissue has a key role in the maintenance and repair of the teeth. It is comprised of the stromal cell population with a high capacity of differentiation into odontoblasts – the main functional cells in dentin regeneration.[22] Previous studies in animal models have shown the association between diabetes and reduced pulp healing capacity.[11] Also, few studies are available on bone marrow-derived stem cells, and the adverse effect of hyperglycemia on their proliferation and differentiation.[23,24] Similarly to our results, Oancea et al.[25] and Yan et al.[26] also revealed the disturbance of dental pulp cell proliferation at high glucose. Recent research indicates that the exposure of bone marrow-derived hematopoietic stem cells to a high glucose-containing medium induces aging, genetic instability and telomere length changes in these cells.[27] However, the precise mechanism of aging induction in these cells remains unclear. There is also further evidence on glucose-induced bone marrow-derived stem cell senescence and the causative role of oxidative-mediated autophagy.[20] While less has been reported on the senescence response in diabetic pulp tissue or cultured pulp cells, our results clearly showed the induction of cellular aging in the cultured pulp cells exposed to long-term hyperglycemic conditions.

Searching for the underlying molecular mechanism, we observed the stimulation of the expression of Wnt1 and beta-catenin. Thereby, it hinted the involvement of the Wnt signaling pathway in glucose-induced pulp cell senescence. Indeed, the Wnt/beta-catenin signaling pathway plays an important role in various cellular processes, such as proliferation, differentiation, migration, survival, apoptosis, and pluripotency.[28,29] The Wnt signaling pathway comprises both the canonical-beta-catenin dependent and the non-canonical-beta-catenin independent pathways, and it is believed that the canonical pathway plays a key role in dentinogenesis and dentin regeneration.[28] In the presence of Wnt ligand and binding to specific cell membrane receptors, cytoplasmic beta-catenin protein is transferred to the nucleus and activates the transcription of many target genes. In the present study, we applied PNU-74564, the binder of beta-catenin, to inhibit the Wnt signaling pathway.[30] Lithium chloride is also known for its role in stabilizing beta-catenin through the inhibition of glycogen synthase kinase 3 beta (GSK3β) activity, and therefore the activation of canonical Wnt signaling.[31] Our results demonstrated that in the presence of beta-catenin inhibitor, the percentage of the senescent cells was reduced, while LiCl significantly enhanced glucose-induced senescence. The data further confirmed the involvement of Wnt signaling, particularly beta-catenin, in the progression of diabetic-induced cellular aging. Regarding the association between Wnt signaling and senescence, Liu et al. through in vitro and in vivo studies revealed that the activation of canonical Wnt-signaling triggers senescence in primary mouse embryonic fibroblasts and epithelial cells.[32] Another study on thymocytes showed that the transgenic expression of beta-catenin induces cell growth arrest, DNA damage and senescence.[33] Also, bone marrow-derived stem cells from systemic lupus erythematosus patients exhibited signs of senescence and showed high activity of beta-catenin.[34] Although there are some other studies which indicate the role of beta-catenin in the induction of cell proliferation.[35,36]

Further detailed clinical studies are essential to assess the pulp tissue proliferation rate and the level of beta-catenin in diabetic patients. Based on the current results, Wnt signaling might be the potential target for the inhibition of the senescence response in the hyperglycemic condition, suggesting the development of bioactive materials applied in pulp capping that would be specific for diabetic patients.

Conclusions

The results of the present study showed that the diabetic condition disturbed pulp cell proliferation and induced the senescence phenotype, which is mediated by beta-catenin/Wnt signaling. Such changes might compromise the healing and regenerative capacity of diabetic pulp. Based on the results of the current study and previous research, it is suggested that differential approaches in endodontic treatment need to be considered for diabetic pulp. Knowledge on the molecular mechanism of diabetic-induced senescence and targeting the key regulatory protein will help us find new therapeutic approaches in endodontic treatment for diabetic patients.

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Original papers

Evaluation of interleukin-1 beta and the ratio of interleukin-1 beta to interleukin-1 receptor antagonist in gingival crevicular fluid during orthodontic canine retraction

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Abstract

Background. Orthodontic tooth movement (OTM) is a complex phenomenon mediated by cytokines, of which interleukin-1 beta (IL-1β) is potently involved in the remodeling of the periodontal ligament (PDL) and bone. Whether the pattern of IL-1β release differs at the sides of tension and compression is not yet clarified.

Objectives. The aim of the present study was to evaluate the level of IL-1β and the ratio of IL-1β to interleukin-1 receptor antagonist (IL-1RA) in gingival crevicular fluid (GCF) at the tension and compression sides during orthodontic canine retraction.

Material and methods. Seventeen patients scheduled for orthodontic treatment with bilateral extraction of maxillary first premolars and canine retraction were enrolled. Tooth 2.3 was retracted, teeth 1.3 and 3.3 served as controls. Gingival crevicular fluid samples were collected from the tension and compression sides of each tooth at baseline (before the 1st activation – day 0) and at days 2 and 7, and then again before the 2nd activation (day 28) and at days 30 and 35. The levels of IL-1β and IL-RA were evaluated with the enzyme-linked immunosorbent assay (ELISA).

Results. After the 1st activation, a statistically significant increase in the level of IL-1β was observed at teeth 2.3 ($p < 0.03$ mesially and $p < 0.05$ distally) and 1.3 ($p < 0.05$ mesially and distally), both at the tension and compression sides. The 2nd activation resulted in a gradual increase in the IL-1β level at both canines; however, statistical significance was reached only for tooth 2.3 ($p < 0.05$ mesially and $p < 0.02$ distally). In terms of the IL-1β/IL-1RA ratio, a significant increase was observed only at the compression side of the experimental tooth ($p < 0.01$).

Conclusions. An increase in the IL-1β level in GCF was observed both at the tension and compression sides of the actively retracted canine 2.3 as well as the contralateral canine 1.3; a significant rise in the IL-1β/IL-1RA ratio was noted only at the compression side of the experimental tooth 2.3, indicating the zone of active bone resorption.

Key words: interleukin-1 beta, orthodontic tooth movement, canine retraction

Introduction

Orthodontic tooth movement (OTM) is based on the periodontal ligament (PDL) and alveolar bone remodeling induced by an external force exerted by an orthodontic appliance.[1] Sterile inflammation is the essence of this biological response.[2] Migrating immune cells and native cells residing in PDL synthesize and release a plethora of cytokines, growth factors, and metabolites of arachidonic acid.[3,4] Although not fully elucidated, their hypothesized role is to mediate tooth movement by promoting the differentiation, maturation and activation of cells responsible for bone remodeling.[5,6]

Many studies have shown elevated levels of several inflammatory cytokines, such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor alpha (TNF-α), in gingival crevicular fluid (GCF) upon the application of the orthodontic force to the tooth.[7–9] Among those, Il-1β, a key mediator in a variety of activities in immune and acute-phase inflammatory responses,[10] seems to be one of the most potent mediators in the process of periodontal tissue remodeling at the initial stages of OTM.[11,12] Its major role is to activate osteoclasts and promote their fusion and survival.[11,12] It has been shown that upon the application of the retracting force to the maxillary canine, the level of IL-1β in GCF at the distal aspect of the tooth elevates, reaching its peak upon 24–72 h.[13,14] Also, a positive correlation between the Il-1β level in GCF and the rate of OTM has been described.[15,16] In vivo, the biological activity of IL-1β is controlled by a naturally occurring antagonist of its receptor, interleukin-1 receptor antagonist (IL-1RA). The application of mechanical stress to the tooth is related not only to an absolute increase in IL-1β secretion, but also to an elevated value of the IL-1β/IL-1RA ratio.[15] Interleukin-l beta also stimulates the production of IL-6, which, just like IL-1β, expresses synergistic activity.[11]

It is well documented that pro-inflammatory cytokines are involved in bone resorption and the inhibition of bone formation.[15] Thus, they facilitate OTM by acting particularly at the pressure side.[11] Probably, a distinct subset of cytokines plays a role in bone apposition at the side of tension. Within these premises, the majority of studies on canine retraction evaluated the cytokine content of GCF only at the side of compression.[13,17] The pattern of their release and their potential role in bone apposition at the tension side are not yet clarified. Therefore, the aim of the present study was to evaluate the GCF level of IL-1β and the ratio of IL-1β to IL-1RA at the compression and tension sides during OTM (canine retraction) in adolescents. The null hypothesis of the study was that there is no difference between the tension and compression sides in terms of either the concentration of IL-1β or the IL-1β/IL-1RA ratio in GCF.

Material and methods

Study population

Seventeen patients (8 males and 9 females) aged from 13 to 18 years (mean age: 16 ±4 years) scheduled to start orthodontic treatment at the Department of Orthodontics, Dental University Clinic in Kraków, Poland, were enrolled to the study. The participants had to meet the following inclusion criteria: 1. no systemic diseases; 2. no use of systemic antibiotic therapy within 2 weeks prior to inclusion; 3. no use of systemic anti-inflammatory drugs within 1 week prior to inclusion; 4. good periodontal health (i.e., the bleeding on probing index (BOP) value not exceeding 10%, no probing depths exceeding 2 mm and no bone loss in the radiographic examination); and 5. the necessity for canine retraction in the course of orthodontic treatment. Ineligibility with respect to any of the inclusion criteria and/or no consent to the enrollment to the study, expressed by either the patient and/or the parents, constituted the basis for exclusion. Among the patients of the study group, the following malocclusions were present: maxillary and mandibular crowding (9 patients); disto-occlusion (1 patient); disto-occlusion with maxillary and mandibular crowding (5 patients); an open bite (1 patient); and the agenesis of maxillary lateral incisors (1 patient).

The study protocol was reviewed and approved by the Bioethical Committee (approval No. KBET/466/B/2003). All patients and their parents gave written informed consent to participation upon receiving detailed explanation of the aim and course of the study.

Course of orthodontic treatment

Careful anamnesis, physical examination, analysis of cast models, orthopantomograms (OPGs), and cephalometric radiographs were carried out for each patient. The treatment plan for the included patients, except for the one with the agenesis of lateral incisors, involved the extraction of maxillary first premolars. All patients were scheduled for maxillary canine retraction. Upon the extractions of premolars, orthodontic separators were placed in the maxillary arch. One week later, bands were bonded on the maxillary first molars. Metal brackets (Victory Series™ MBT .022"; 3M Unitek, Maplewood, USA) were bonded onto the teeth in the upper arch. Additionally, all patients received transpalatal arches (TPAs) bended from a 0.9-millimeter wire in order to prevent the mesial movement of first molars during active canine retraction. The retraction of canines was performed according to an individual orthodontic treatment plan based on a careful examination of each patient, and the analysis of OPGs, cephalometric radiographs and plaster models.

In each patient, the retraction was firstly initiated for the upper left canine (tooth 2.3), which was the experimental tooth. The retraction of the contralateral canine (tooth 1.3) was delayed by 2 months, until the end of the experimental procedure (GCF collection – see below). Teeth 1.3 and 3.3 served as controls.

All patients received also orthodontic treatment in the lower arch by means of fixed orthodontic appliances upon the completion of the experimental procedure in order to ensure proper occlusal relations. Orthodontic treatment lasted around 2 years in each patient. Upon the completion of the treatment, all patients were provided with a removable splint for the maxillary arch and a fixed orthodontic retainer in the mandibular arch.

Experimental procedure – recorded parameters

In each patient, GCF was collected from the mesial and distal aspects of the experimental tooth 2.3 and the control teeth (1.3 and 3.3). The GCF collection was performed initially before the 1st activation of the appliance (day 0), and 48 h (day 2) and 168 h (day 7) after the 1st activation. After 4 weeks, GCF was sampled again before the 2nd activation (day 28), and 48 h (day 30) and 168 h (day 35) after the 2nd activation. The study protocol is presented on the flowchart (Fig. 1).

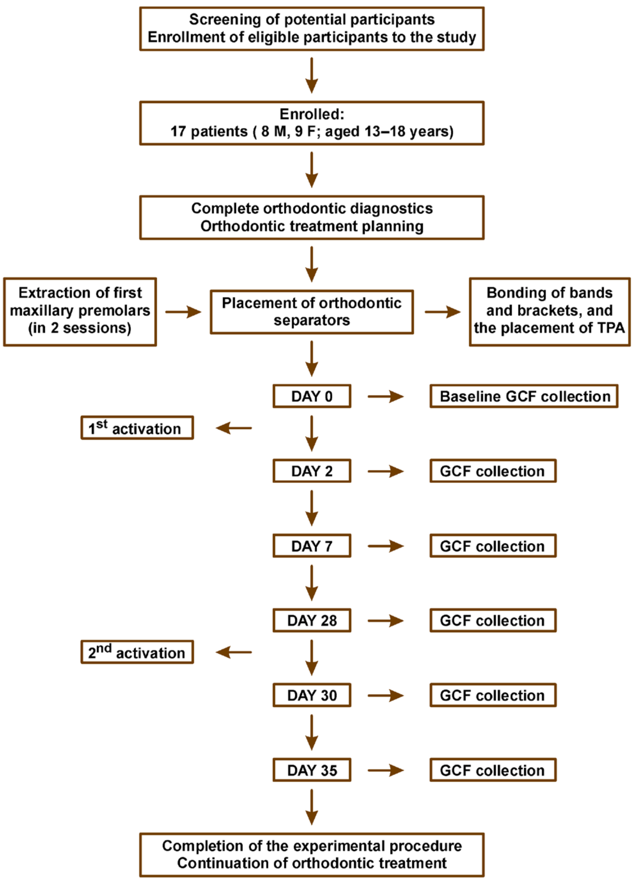

Fig. 1. Flowchart of the experimental protocol

M – males; F – females; TPA – transpalatal arch; GCF – gingival crevicular fluid.

GCF collection

Each patient received detailed oral hygiene instructions prior to the placement of brackets, and was motivated to reinforce oral hygiene throughout the entire experimental period in order to minimize the influence of gingival inflammation on the cytokine levels. Additionally, the patients were instructed to rinse their mouth with 0.1% chlorhexidine mouthwash for 1 min twice a day, for 2 weeks prior to the GCF collection procedure.

At the appointments for the GCF collection, the sampling teeth were isolated with cotton rolls and suction, cleaned with cotton pellets soaked in distilled water and gently dried with air from an air syringe. Gingival crevicular fluid was collected on sterile absorbent filter-paper strips (PerioPaper® GCF Collection Strips; Oraflow, Plainview, USA). In brief, the PerioPaper strip was grasped by holding the orange handle with pliers and its white portion was gently inserted to the gingival crevice until a minimum of resistance was felt, and it was kept in situ for 30 s. During each appointment, a maximum of 3 strips were inserted at each side at 1-minute intervals in order to collect a specified volume of fluid, sufficient for the biochemical analysis. In total, a maximum of 3 separate strips were collected from the mesial, and 3 from the distal aspect of the experimental tooth 2.3. Similar procedures were performed at the control teeth (1.3, 3.3) in each patient at each time-point of the study. Directly upon collection, the volume of GCF on each strip was determined using Periotron 8000® (Oraflow). Prior to measurements, the instrument was carefully calibrated, and a standard calibration curve was constructed using a range of volumes of human serum of known biochemical compounds' concentrations. Following volume determination, the strips were placed in sterile cryo-tubes and kept in −70°C until the biochemical analysis.

Biochemical analysis

When the process of GCF collection was completed in all patients, the samples were defrosted, and the strips collected from the same aspect (either mesial or distal) of the same tooth at the same time-point were pooled together in a vial. The fluid was eluted from the strips as follows: 110 μL of Tris-HCl buffer (pH 7.5) was added to each vial and the vials were shaken on an orbital shaker for 10 min; subsequently, the vials were centrifuged at 1,500 g for 5 min. The supernatant was placed in a fresh vial. This procedure was repeated to gather the proper volume, and the end volume of the eluate was 200 μL.

The concentration of IL-1β and IL-1RA in the eluates was determined by means of the immunochemical method – the enzyme-linked immunosorbent assay (ELISA) (BioSource, Ottignies-Louvain-la-Neuve, Belgium; R&D Systems, Minneapolis, USA, respectively). Additionally, the total protein concentration was determined spectrophotometrically.

Data analysis

Patient enrollment, and all the procedures related to orthodontic treatment and the GCF collection were performed by one examiner (S.M.).

For each GCF sample, the degree of eluation with Tris-HCl buffer was calculated based on the initial sample volume (measured by means of Periotron 8000) and the known volume of the added buffer. This dilution was taken into account when calculating the concentrations of cytokines and the total protein concentration. The concentrations of cytokines were calculated in relation to the protein concentration [pg/μg]. The obtained results were presented as mean (*M*) ± standard deviation (*SD*) for the mesial and distal sides of each examined tooth. Changes in the cytokine levels for each tooth at different time-points were compared by means of Student's *t* test upon the confirmation of normal distribution. The level of statistical significance was set at $p < 0.05$.

Results

All 17 patients completed the study. None of the study participants exhibited gingival inflammation during the experimental period.

GCF total protein concentration

Figure 2 presents the total protein concentration in GCF at the experimental (2.3) and control (1.3 and 3.3) teeth. An increase in the total protein concentration in GCF was observed at all evaluated teeth upon the bonding of braces, i.e., the 1st application of the orthodontic force and the activation of the experimental canine. This increase reached statistical significance with respect to baseline at days 2 ($p < 0.020$) and 7 ($p < 0.001$) for the mesial aspect of the control tooth 1.3, and at day 7 for the mesial aspect of tooth 2.3 ($p < 0.005$). Of note, a rise in total protein in GCF was also significant for the control tooth 3.3, reflecting the general response within the oral cavity to the occurrence of the orthodontic force. Following the 2nd activation, the level of protein dropped, and this effect was also noticeable for all evaluated teeth.

GCF concentration of IL-1β

The GCF concentration of IL-1β at the experimental (2.3) and control (1.3 and 3.3) teeth is presented in Fig. 3. Upon the 1st activation of the retracted canine 2.3, a statistically significant increase in the level of IL-1β in relation to total protein was observed at day 2, both for the mesial and distal aspect of this tooth ($p < 0.030$ and $p < 0.050$, respectively). At day 7, a drop in IL-1β/total protein was observed at both sides; however, it reached statistical significance only at the mesial aspect ($p < 0.010$). Following the 2nd activation, a significant increase in IL-1β/total protein was observed at day 7 (experimental day 35), mesially and distally ($p < 0.050$ and $p < 0.020$, respectively).

Similar changes in the amount of IL-1β in relation to the total protein content in GCF were observed at the control

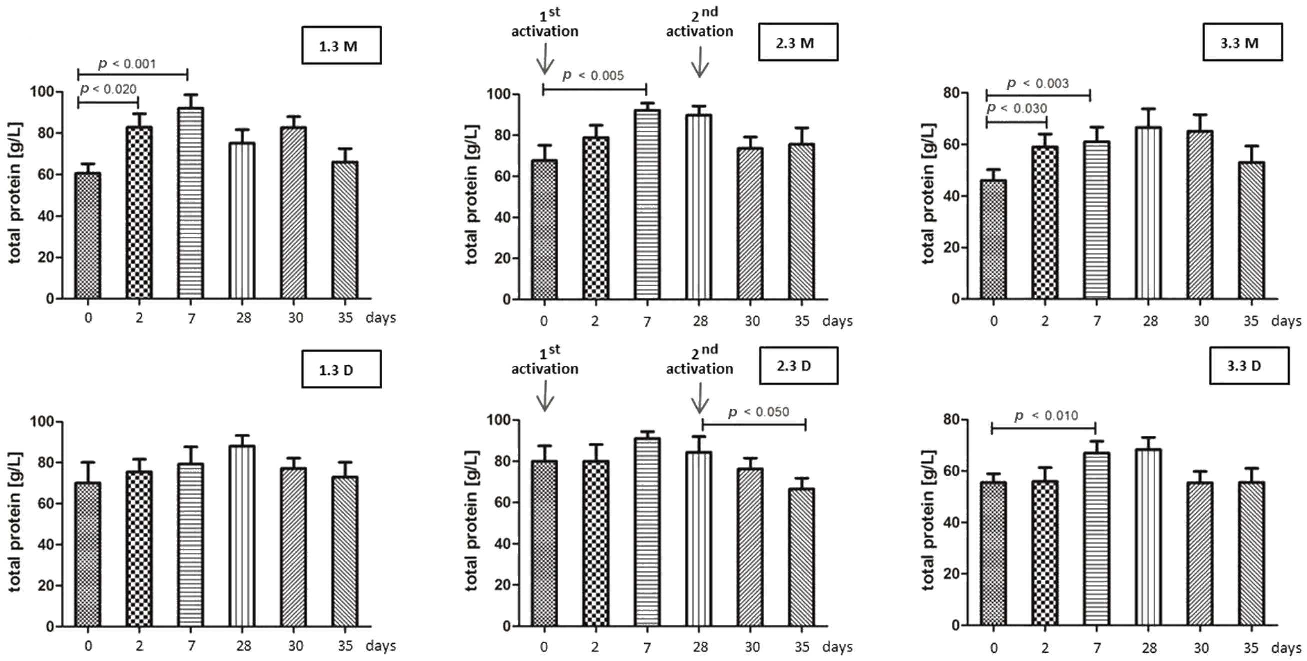

Fig. 2. Mean total protein concentration at the evaluated teeth [g/L]

Error bars indicate standard deviation (*SD*); symbols in the upper right frames at each chart represent tooth number (1.3, 2.3, 3.3) and the evaluated side (M – mesial, D – distal).

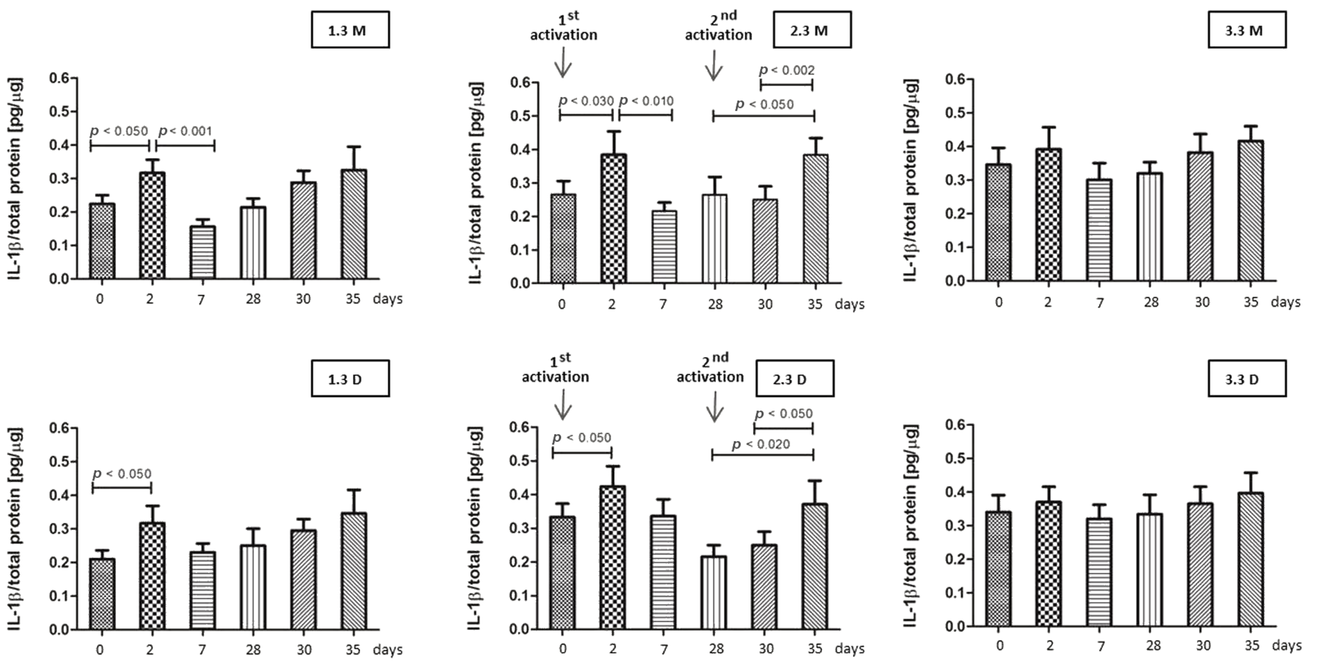

Fig. 3. Mean concentration of interleukin-1 beta (IL-1β) in relation to total protein [pg/µg]

Error bars indicate standard deviation (*SD*); symbols in the upper right frames at each chart represent tooth number (1.3, 2.3, 3.3) and the evaluated side (M – mesial, D – distal).

tooth 1.3, i.e., a rapid and significant increase on day 2 after the 1st application of the orthodontic force ($p < 0.050$ for both the mesial and distal aspect) with a subsequent, albeit not significant (except for the mesial aspect, with $p < 0.001$), decrease at day 7, and a tendency to a gradual increase throughout the 7-day period upon the 2nd activation.

No significant changes in IL-1β/total protein were observed for the control tooth 3.3.

GCF IL-1β to IL-1RA ratio

The ratio of IL-1β to IL-1RA in GCF at the experimental (2.3) and control (1.3 and 3.3) teeth is presented in Fig. 4.

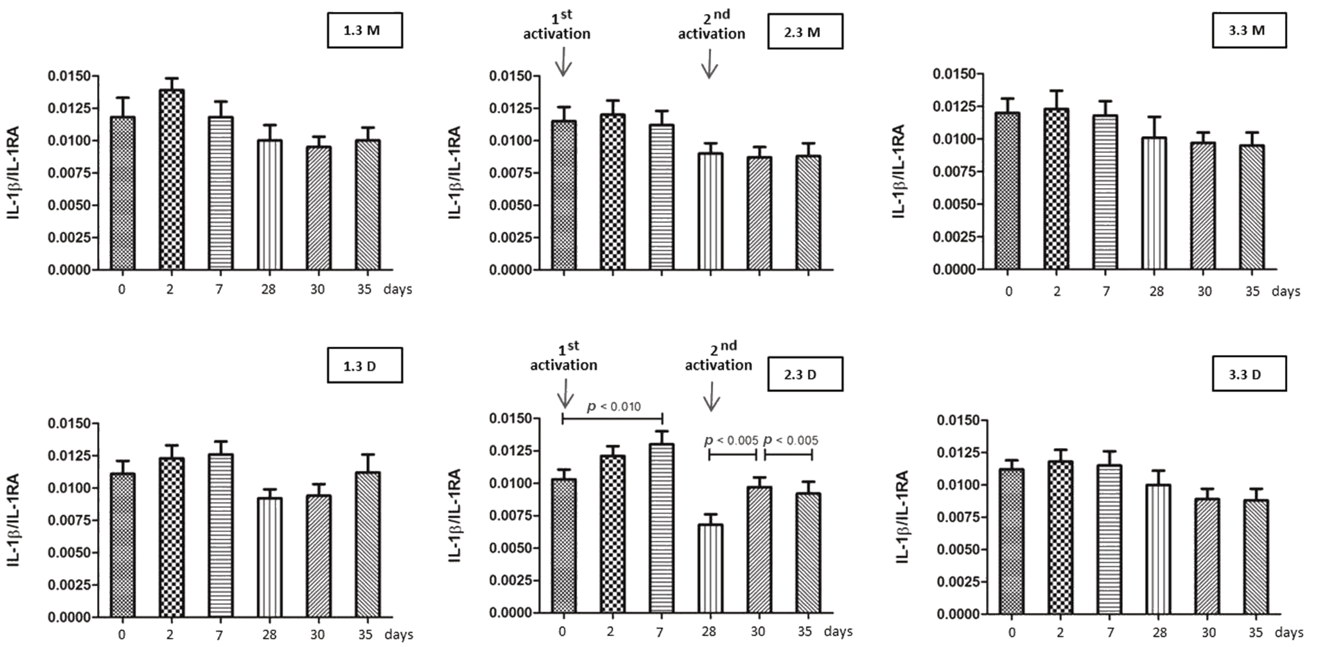

Fig. 4. Mean IL-1β to interleukin-1 receptor antagonist (IL-1RA) ratio

Error bars indicate standard deviation (*SD*); symbols in the upper right frames at each chart represent tooth number (1.3, 2.3, 3.3) and the evaluated side (M – mesial, D – distal).

Interestingly, statistically significant changes in this ratio were observed only for the distal aspect, i.e., the compression side, of the experimental tooth. Upon the 1st activation, a gradual increase in IL-1β/IL-1RA was noted throughout the following 7 days, and it reached statistical significance at day 7 ($p < 0.01$). Following the 2nd activation, a rapid rise in IL-1β/IL-1RA took place at day 2 (experimental day 30) ($p < 0.005$), and the level of this relation remained relatively stable until day 7 (experimental day 35). In turn, no significant changes in IL-1β/IL-1RA were observed at the mesial aspect, i.e., the tension side, of the experimental tooth 2.3 as well as at the control teeth (1.3 and 3.3), both mesially and distally.

Discussion

The application of the orthodontic force to a tooth results in a series of orchestrated cellular and molecular events, leading to the remodeling of periodontal tissues.[18] Within this remodeling, 2 distinct processes take place simultaneously at the opposite sides of the moved tooth, i.e., bone resorption at the pressure side and bone deposition at the tension side. A bunch of cytokines, of which pro-inflammatory IL-1β is the most potently associated with resorption, mediate these phenomena. Indeed, IL-1β induces the expression of the receptor activator of the nuclear factor in osteoblasts and PDL cells, and stimulates the differentiation of osteoclast precursors as well as the fusion, survival and activation of matured osteoclasts.[2,16,19] Hence, its role seems of particular importance at the compressed areas of PDL, rather than at the sides where tension occurs. Thus, IL-1β has been proposed as a biological marker of tissue remodeling. The biological effects of IL-1β are, however, controlled by a naturally occurring antagonist of its receptor, IL-1RA.[20] Thus, monitoring the IL-1β/IL-1RA ratio gives the most credible view on the intensification of resorptive processes. Based on this premise, the aim of the present study was to evaluate the level of IL-1β and the IL-1β/IL-1RA ratio in GCF at the initial stages of OTM (canine retraction), at the sides of compression and tension.

An increase in the total protein content in GCF was observed within the 1st week upon the 1st application of the orthodontic force at the experimental tooth as well as at the control teeth. This is an interesting observation and suggests that the introduction of an orthodontic appliance to the oral cavity, although initially bonded only in one dental arch, induces a generalized cascade of events in terms of upregulation of gene expression and protein synthesis in periodontal tissues.

At the sides around the retracted canine 2.3, an increase in the IL-1β concentration was observed both mesially and distally, following the 1st (a rapid rise within the first 48 h, with a drop toward a level similar to baseline at day 7) and the 2nd activation (a gradual increase over the following 7 days). Interestingly, a significant increase in the IL-1β/IL-1RA ratio was noted only at the distal aspect of tooth 2.3, indicating that the compression side of the retracted canine is characterized by the highest potential of bone resorption. Although at the mesial aspect of the retracted canine, the level of IL-1β was elevated after the activations, the IL-1β/IL-1RA ratio exhibited stable values, indicating controlled bone metabolism without a tendency toward resorption.

An increase in the IL-1β concentration was observed also at the control canine 1.3. This can be attributed to the fact that the orthodontic force was distributed within the whole upper dental arch due to the presence of a fixed orthodontic appliance and the retraction of tooth 2.3, eliciting a biologic response at all the teeth incorporated to the appliance. However, no significant alterations of the IL-1β/IL-1RA ratio were noted at this tooth.

No significant changes in either the IL-1β concentration or the 1β/IL-1RA ratio were noted at the control tooth 3.3.

A similar pattern of the upregulation of this cytokine at the side of compression in the course of canine retraction has been reported in other studies.[13,14,21,22] Researchers described an elevation of the IL-1β concentration in GCF in the early phases of canine distalization, with peaks at either 24 h[13,21,22] or 72 h,[14] and with a subsequent decrease toward baseline levels at 168 h. Among the above-mentioned studies, only Uematsu et al. used both the contralateral and antagonist canines as controls,[21] whereas in the other investigations, only the tooth contralateral to the retracted one served as a control.[13,14,22] Of note, Uematsu et al. did not observe an elevation of the IL-1β concentration for the contralateral canine,[21] which was instead shown in our study. In all those studies, the concentration of Il-1β was measured either exclusively at the compression side[13] or without side specification,[14,21,22] contrarily to our study, in which the cytokine level was specifically assessed both at the compression and tension sides. In turn, Iwasaki et al. compared the IL-1β level at the mesial and distal sides of the retracted canines and reported a higher increase at the compression side.[15] The activity of IL-1β remains under the control of IL-1RA. It has been shown that lower values of the IL-1RA concentration and higher values of the IL-1β/IL-1RA ratio are associated with a higher velocity of tooth movement during orthodontic treatment.[23] An investigation described higher values of IL-1β/IL-1RA at the distal aspect of the retracted canine than at its mesial aspect,[9] which is in accordance with the outcomes of the present study.

Based on the current knowledge on the effects of the IL-1β upregulation on the velocity of tooth movement, attempts have been made to further increase the synthesis of IL-1β in order to accelerate OTM. Varella et al. showed that the application of low-level laser therapy with a gallium-aluminium-arsenide semiconductor laser during orthodontic treatment contributes to increasing the level of IL-1β in GCF and significantly accelerates tooth translation.[16]

The application of the orthodontic force induces the upregulation of a plethora of pro-inflammatory cytokines and enzymes, which act synergistically in the process of tooth movement. Ren et al. pointed that also IL-6, IL-8 and TNF-α play a significant role at the early stages of tooth movement.[9] Grant et al. compared cytokine profiles at the compression and tension sides during maxillary canine retraction and noted that while both sides were characterized by increases in the concentration of IL-1β, IL-8, TNF-α, and matrix metallopeptidase-9 (MMP-9), they differed in terms of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) and tissue inhibitor of matrix metalloproteinase-2 (TIMP-2) (expressed in higher values at the tension side), and receptor activator of nuclear factor kappa-B ligand (RANKL) (expressed in a higher value on the compression side).[24] The distal movement of the canine has also been related to the rise in the activity of alkaline phosphatase at the initial stages of treatment.[25]

To the best of the authors' knowledge, this is the only study in which the GCF concentration of IL-1β as well as IL-1β/IL-1RA were evaluated at both sides, i.e., compression and tension, and compared to the contralateral and antagonist canines. Although increased IL-1β synthesis was observed at the compression and tension sides of the retracted and contralateral canines, a significant imbalance between the concentration of this cytokine and its antagonist was observed exclusively at the compressed side of the retracted tooth.

With respect to the clinical relevance of the presented research findings, it should be noted that measuring the levels of IL-1β and IL-1RA could potentially be applied for the determination of proper levels of orthodontic forces in order to obtain desirable velocity of tooth movement. Predicting OTM requires controlled bone resorption, so the rate of the IL-1β and IL-1RA synthesis might be an important indicator of this phenomenon. To this end, future studies may focus on determining the relation between IL-1β/IL-1RA and the rate of OTM.

Limitations

The results of this study should, however, be carefully considered in the light of several limitations. The main shortcoming is a relatively small study group. The fact that the number of participants was low might have influenced the magnitude of significance for the observed associations. Consequently, the findings of this research should be interpreted cautiously. Secondly, we did not measure the rate of canine retraction upon the application of the orthodontic force, so we were not able to determine whether an increase in the IL-1β level and in the IL-1β/IL-1RA ratio indeed correlated with the velocity of this process. It may also be debatable if differences on the biochemical level between the pressure and tension sides of a single tooth can be precisely shown due to close proximity. Therefore, further clinical studies on larger populations and with well-designed protocols are essential to elucidate the molecular mechanisms responsible for OTM.

Conclusions

Based on the obtained results, it can be concluded that the protein content in GCF increases in response to the introduction of the orthodontic force and this response takes place in the arch incorporated to the orthodontic appliance as well as in the opposite arch. The application of the orthodontic force to a tooth leads to a rapid increase in the IL-1β level in GCF at the early stages of treatment, at both compression and tension sides, with the subsequent normalization of its concentration. The application of the distalizing force to a tooth is related to a rise in the IL-1β/IL-1RA ratio at the compression side, indicating active bone resorption.

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Original papers

Effect of the use of a surgical guide on heat generation during implant placement: A comparative in vitro study

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Abstract

Background. Heat generation is considered a decisive factor in the occurrence of bone necrosis during implant placement, which can happen when the temperature exceeds a threshold of 47°C for 1 min. The use of a surgical guide to aid implant placement has gained popularity in the last few years. Whether it increases the risk of bone necrosis is still debatable.

Objectives. The aim of the present study was to compare heat generation during implant placement with and without the use of a surgical guide.

Material and methods. The study sample consisted of 80 measurement sites placed near 40 dental implant sockets, which were prepared on 10 bone-like dental models. These models were divided into 5 models for the conventional method group and 5 models for the surgical guide group. Each model had 4 implant sockets prepared, and then two 1-millimeter-wide holes were drilled <1 mm away from the socket on the opposite sides of the implant socket to be used as temperature measurement sites. The diameter of the drill was standardized to 2.2 mm, and 4 different drill lengths were used (6, 8, 10, and 12 mm). The data was analyzed using the SPSS for Windows software, v. 13.0. A *p*-value of <0.05 was deemed statistically significant.

Results. Significant differences were found in heat generation between the conventional group (41.07°C) and the surgical guide group (42.97°C) ($p < 0.05$). Significant changes in temperature were recorded after drilling, regardless of the method used ($p < 0.05$). Moreover, the length of the drill was associated with temperature changes, with longer drills generating more heat ($p < 0.05$).

Conclusions. Within the limitations of this study, the use of a surgical guide resulted in higher temperatures as compared to the conventional method of implant placement. However, the highest recorded temperature was far below the threshold for bone necrosis.

Key words: dental implants, bone, temperature

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Introduction

Dental implants have become an essential part of routine dental treatment to replace lost teeth. The conventional dental implant placement procedure involves making a surgical incision into the gingiva on the crest of the alveolus and elevating a mucoperiosteal flap to access the bone underneath it, then placing the dental implants, replacing the flap, and finally suturing the wound.[1,2]

Recently, flapless dental implant placement has gained popularity as an alternative to the conventional dental implant placement procedure with many advantages, such as being less invasive, possibly causing less postsurgical discomfort, shortening the duration of the surgical procedure, and minimizing changes that occur in the alveolar crest. This might be attributed to a relatively small surgical incision and not raising a mucoperiosteal flap.[3–5]

Moreover, various computer-guided systems are now available to help in making a precise three-dimensional (3D) diagnosis, and the programs included in these systems aid in accurately transferring the locations of virtual implants from the computer to appropriate sites in the patient's mouth. The necessity for this accuracy has become more prominent, especially after the adoption of computer-guided systems in flapless implantation procedures.[6]

The effectiveness of surgical guides remains a topic for scientific research, especially given what some studies suggest regarding the effectiveness of using surgical guides with irrigation cooling. This is a crucial point, as exposing bone tissue to a temperature of 47°C for a full minute is considered a threshold for causing irreversible osteonecrosis, which necessitates taking measures to avoid subjecting the bone to mechanical or thermal damage.[7,8]

Therefore, the aim of our study was to compare changes in bone temperature while preparing the implant site with the use of a surgical guide and by means of the conventional method of implantation. Moreover, we assessed the effects of using various drill lengths on heat generation.

Material and methods

A comparative laboratory study was conducted to assess an increase in temperature when using either the conventional method or a surgical guide for dental implant placement. The study was carried out between December 2019 and March 2020.

The study sample consisted of 80 measurement sites placed near 40 dental implant sockets, which were prepared on 10 artificial-bone blocks similar to human D2 bone (solid rigid polyurethane foam; Alexandria Industries, Taipei, Taiwan). These models were divided into 5 models for the conventional method group and 5 models for the surgical guide group (Fig. 1). Each model had 4 implant sockets prepared, and then two 1-millimeter-wide holes were drilled 1 mm away from the socket on the opposite sides of the implant socket to be used as temperature measurement sites (Fig. 2). The holes were drilled using Peeso reamers (Rogin Dental, Shenzhen, China) on a contra-angle handpiece (NSK, Tokyo, Japan) (Fig. 3).

Each bone block was placed in an aqueous medium of a temperature similar to body temperature (37°C),[9] then a thermometer (Thermocouple Type K device; Danoplus, Hong Kong, China) was placed inside the measurement sites and the temperature was recorded (Fig. 4). The implant motor (X-Cube implant motor system; Dentis, Daegu, South Korea) was set to 1,200 rpm, 30 N and 85% irrigation for the preparation of all implant sites.

Drilling was started for the implant socket with a 6-millimeter-long bur of a diameter of 2.2 mm (Dentis) and the temperature was recorded immediately after drilling in the temperature measurement sites. The temperature was also recorded before and immediately after drilling with the use of 8-, 10- and 12-millimeter-long drills. The same procedure was applied in the surgical guide group, following the insertion of the customized surgical guide.

Having collected the data, it was entered and analyzed using the SPSS for Windows software, v. 13.0 (SPSS, Inc., Chicago, USA). The statistical analysis was conducted

Fig. 1. Bone model with the surgical guide mounted

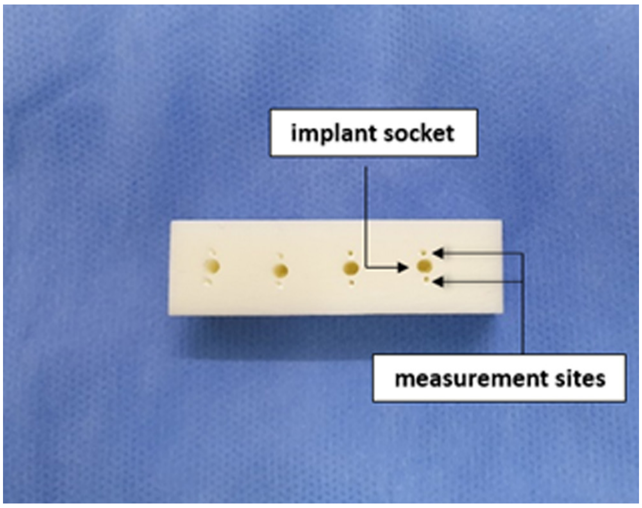

Fig. 2. Bone model showing the implant sockets and the temperature measurement sites

Fig. 3. Laboratory materials (from left to right): a battery, Peeso reamers, a contra-angle handpiece, cords, a thermometer, bone blocks, water, and a surgical guide

at the significance level set at 0.05 ($p < 0.05$). The analysis of variance (ANOVA) was used to determine the existence of statistically significant differences when comparing more than 2 independent variables, and the Bonferroni correction was used to perform multiple comparisons. The mean temperature of all measurement sites was used to study the different variables, except for the temperature differences between the 2 measurement sites, where the comparison was made between the means of the opposite sides.

Fig. 4. Temperature recorded in 2 measurement sites

Results

The study sample consisted of 80 measurement sites for 40 dental implant locations (orthopedic pits), which were prepared on 10 artificial-bone blocks.

Table 1 shows the sample temperature before and immediately after drilling with regard to the following variables: implant placement method; drill length; and measurement site.

The mean temperature in the surgical guide group was 38.14°C before drilling and became 42.49°C after drilling whereas in the conventional method group, the mean temperature was 37.64°C before drilling and 41.07°C after drilling.

Table 1. Descriptive statistics of the variables in terms of temperature [°C] before and after drilling

Variable		Status	Counts	*M*	*SD*	Min	Max
Implantation method	surgical guide	before drilling	40	38.14	1.14	35.3	39.8
	conventional		40	37.64	0.88	34.9	38.9
	surgical guide	after drilling	40	42.49	1.87	39.5	45.4
	conventional		40	41.07	1.98	37.1	43.8
Drill length	6-millimeter	before drilling	20	36.47	0.89	34.9	38.5
	8-millimeter		20	38.09	0.43	37.2	38.9
	10-millimeter		20	38.39	0.48	37.4	39.3
	12-millimeter		20	38.61	0.60	37.6	39.8
	6-millimeter	after drilling	20	39.19	1.29	37.1	41.3
	8-millimeter		20	41.22	0.60	40.3	42.2
	10-millimeter		20	42.81	0.83	41.3	44.0
	12-millimeter		20	43.91	1.14	41.5	45.4
Measurement site	site A	before drilling	40	37.84	1.03	35.3	39.6
	site B		40	37.94	1.07	34.9	39.8
	site A	after drilling	40	41.65	2.12	37.1	45.4
	site B		40	41.91	1.98	37.2	45.3

M – mean; *SD* – standard deviation; min – minimum; max – maximum.

It was also noted that the temperature increased with an increasing drilling depth, with the average temperature after drilling of 39.19°C following the use of a 6-millimeter drill as compared to 43.91°C following the use of a 12-millimeter drill.

No significant differences in temperature were observed between the opposite sides of the implant socket.

The ANOVA test was performed to study the effects of the pre-drilling temperature, the implantation method, the length of the drill, and the measurement site on the temperature recorded after drilling (Table 2).

Table 2. Association between the variables and the recorded temperature (ANOVA)

Variable	*F*-value	*p*-value	η^2-value	Severity of effect
Implantation method	66.25	0.001*	0.513	mild
Drill length	64.63	0.001*	0.755	strong
Measurement site	2.59	0.113	–	–
Pre-drilling temperature	10.09	0.002*	0.138	weak

* statistically significant.

Table 2 highlights that no statistically significant difference was found between the temperature measurement sites on both sides of the implant socket (p = 0.113).

As for the remaining independent variables, significant differences in the temperature after drilling were found between all of them.

Table 3 shows the mean (M) and standard error (SE) values for the temperature recorded following the use of the 2 surgical implant placement methods and different length drills, on both sides of the implant socket.

Table 3. Estimated mean (*M*) and standard error (*SE*) temperature values [°C] for each variable

Variable		*M*	*SE*
Implantation method	surgical guide	42.38	0.10
	conventional	41.18	0.10
Drill length	6-millimeter	39.76	0.22
	8-millimeter	41.13	0.14
	10-millimeter	42.60	0.15
	12-millimeter	43.62	0.16
Measurement site	buccal	41.67	0.09
	lingual	41.89	0.09

The multiple comparison test was conducted using the Bonferroni method on the mean temperature values after drilling to compare various drill lengths, as shown in Table 4.

Significant differences in heat generation were found between any 2 drill lengths used.

Table 4. Multiple comparison between different drill lengths using the Bonferroni correction

Hole depth [mm]		Difference between the groups	*SE*	*p*-value
6	8	−1.37	0.28	0.001*
	10	−2.84	0.31	0.001*
	12	−3.86	0.33	0.001*
8	10	−1.47	0.19	0.001*
	12	−2.49	0.20	0.001*
10	12	−1.02	1.19	0.001*

* statistically significant.

Discussion

The amount of heat generated by and transmitted from burs to the bone during drilling while preparing the implant socket depends on several factors, including the speed of rotation of the drill, the number of drills used, the design and shape of the drill, the depth of drilling, the cutting edge of the drill, and the use of internal or external irrigation. To sum up, what is important with regard to heat generation are the cooling mechanisms and the forces applied during the drilling process.[9] Therefore, if a surgical guide is to be used, it should have cooling channels to reduce an increase in bone temperature. This is particularly important when drilling is performed in harder bone, such as D1 or D2, since the use of a surgical guide may reduce the effectiveness of irrigation.[10]

The results of this study showed that there was a statistically significant effect of each of the implant placement method and drill length as well as of the temperature before drilling on the temperature values after drilling. The fact that a surgical guide hampers the access of the irrigation fluid to the drilling site may explain a significant increase in temperature in case a surgical guide is used for dental implant placement.[7] The rise in temperature which occurs when the first 2.2-millimeter bur is used is due to the need to remove a large amount of bone, which causes friction to the bone during drilling.[11] The clinical temperature values recorded after the bone drilling process are usually lower and safer than those recorded in laboratory studies; this might be due to the fact that the blood circulation within bone tissue dissipates a slight amount of the heat generated during the drilling process.[12]

A slight difference in the mean pre-drilling temperature of 0.5°C was noted. All bone blocks were placed in an aqueous medium of a temperature of 37°C in an attempt to standardize the pre-drilling temperature. However, slight differences can be expected, as the temperature of the medium can be 37 ±0.5°C.

The highest temperature recorded in our study did not reach the threshold for osteonecrosis.

These results align with the results obtained by Misir et al., who conducted their study on bone-like dental models using the Thermocouple Type K device with a drilling speed of 1,500 rpm.[13] The researchers used 2 different systems of drilling burs: in the 1st group, an external irrigation system was used, and in the 2nd group, both internal and external irrigation systems were applied. The authors concluded that drilling the implant socket with the use of a surgical guide resulted in a greater increase in temperature in comparison with the conventional method.[13]

Moreover, the results of the present study are in line with a study by Strbac et al., where it was found that the highest increase in bone temperature was obtained after reaching the deepest point in the hole, and that heat generation was a result of friction between the bone and the drill surface.[14]

Our results are also consistent with those of Kim et al., who conducted their study on models of artificial bone using the Thermocouple Type K device, and made measurements at depths of 1, 5 and 10 mm.[15] The authors showed that the highest internal temperature of the bone was at a depth of 10 mm (44 ±1.09°C).[15]

One of the limitations of this study was that it was done in a laboratory setting, where the recorded temperature may not be identical to that achieved in clinical conditions. Such studies are hard to perform in vivo; there would be some ethical concerns with regard to drilling 2 temperature holes in the patient's mouth, and also there would be anatomical difficulties with placing the probes intraorally. With that said, the highest temperature achieved in the current study was still lower that the threshold for bone necrosis. Therefore, it is the authors' belief that when using a good irrigation system in clinical conditions, lower and safe temperatures are expected due to the added benefit of the blood circulation. Another limitation is measuring the temperature in the temperature holes rather than the implant socket itself, although the closeness of the hole to the implant socket may reduce the amount of temperature discrepancies that might occur.

Conclusions

Within the limitations of this study, we can conclude that using a surgical guide results in a higher temperature as compared to the conventional method of implant placement, as a surgical guide can impede irrigation. However, the observed increase was not statistically significant and the highest recorded temperature was below the threshold for bone necrosis.

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Original papers

Clinical evaluation of the implant survival rate in patients subjected to immediate implant loading protocols

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Abstract

Background. In the past 20 years, several studies and clinical trials have reported similar results for transmucosal implants as compared to submerged implants. Several advantages of immediate loading have been pointed out, such as the reduction of treatment time, trauma reduction, and immediate esthetic and functional improvements.

Objectives. The main objective of this study was to clinically evaluate the implant survival rate in patients with total rehabilitation via implants that underwent immediate loading in the past 5 years.

Material and methods. A cross-sectional, descriptive, observational analysis was conducted. The implant survival rate for an edentulous maxilla or mandible was assessed with regard to the loading protocol by means of a questionnaire and clinical observation. The study included 103 patients with edentulous jaws rehabilitated with fixed prostheses on implants. Each patient received 4–6 implants. In total, 474 implants were placed. Factors such as the implant survival rate as well as biological and prosthetic complications were evaluated and analyzed statistically.

Results. Of the 474 implants initially placed, 458 were considered osteointegrated and 16 were considered lost, which corresponds to a 96.62% implant survival rate. The most common types of failure were prosthetic fractures (46.2%), peri-implantitis (23.1%) and unscrewing (11.5%) in the first 5 years.

Conclusions. The rate of osseointegration for implants placed under immediate loading was extremely high, in accordance with the previously published studies, which led us to conclude that currently, this is a surgical procedure with a high rate of success and high predictability.

Key words: dental implants, osteointegration, primary stability, immediate loading, micromovements

Introduction

In recent decades, dentistry has undergone enormous and continuous evolution. More than 45 years ago, Brånemark demonstrated the ability of natural bone to accept titanium during the period of bone remodeling, thus giving rise to the concept of osteointegration.[1–5]

Classical protocols proposed that implants should not support loads during osteointegration to avoid micromovements, which are considered one of the main risk factors for osteointegration. Thus, the rehabilitation of edentulous patients was performed through a two-stage surgical system. The implants were submerged in soft tissue for a period of 3 months for the mandible and 5–6 months for the maxilla, allowing healing without any occlusal load. The main disadvantages of this technique were the need for a second surgery to expose the implant and place the prosthetic or healing abutment, and a longer period of edentulousness.[6]

In the past 20 years, several studies and clinical trials have reported similar results for transmucosal implants as compared to submerged implants. According to the researchers, it is not necessary to submerge implants under the mucosa during the healing period, which creates an opportunity for an immediate loading protocol.[7]

The definition of an immediate load has evolved, and the most current one was established in 2008 by Esposito et al.[7] According to this definition, a load is applied to the implant up to 1 week after surgery through a provisional restoration, anatomically similar to the final restoration, which is placed later.[8]

Several advantages of immediate loading have been pointed out, such as immediate esthetic and functional improvements, the exclusion of temporary removable prostheses, the prevention of second surgeries, the preservation of soft tissue anatomy, the reduction of treatment costs, easier hygiene due to the reduced number of implants used, and minimizing the need for grafts, since the patient's bone base is used to the maximum extent possible due to the inclination of implants.[9–11]

On the other hand, greater care is needed in patient selection. Therefore, there are contraindications: cases of uncontrolled diabetes; a weakened immune status; blood dyscrasia; and insufficient bone.[10,12]

According to recent studies, implants placed with an immediate load with fixed full-arch prostheses reach very high success rates after several years of follow-up, both in the post-extraction and healed bone, and both in the maxilla and the mandible.[13]

However, Esposito et al., in their latest Cochrane systematic review of loading protocols, concluded that although in selected patients, immediate loading can be performed successfully, trends indicate that the immediately loaded implants fail more often than those following a conventional protocol.[7] In addition, the authors concluded that the topic of immediate loading in toothless jaws is well documented, unlike in the case of dentulous jaws, for which there is less evidence available.[7]

It is well known that good primary stability of the implant is a key condition for the success of immediate loading. This primary stability is influenced by many factors, including local bone quality and quantity, the macrodesign of the implant and the surgical technique. To assess primary stability, it is sufficient to measure the implant insertion torque value; this parameter is easily accessible and is a determinant of success in osteointegration.[14]

Micromovements are one of the main risks that might prevent successful osteointegration. Micromovements greater than 150 μm can compromise the entire process, resulting in the fibrous encapsulation of the implant.[15]

There is a critical micromotion threshold above which fibrous encapsulation prevails over osteointegration. This critical level, however, is not 0 μm, as might be expected. The tolerated micromotion threshold was defined between 50 and 150 μm. In this range of tolerated micromovements, an initial load on the implant surface can even stimulate the newly formed bone to remodel, accelerating the osteointegration process.[15]

Torque values of 30–40 N·cm were generally chosen as the minimum acceptable values for immediate loading. This minimum torque level is important both to guarantee the osteointegration process and to fix the implant–abutment connections through the union screw.[16]

According to Eliasson et al.[17] and Fisher et al.,[18] technical complications often arise when applying an immediate loading protocol. The most common ones are the fracture of the prosthesis, the loosening of the abutment screws and the adjustments of the contour of the prosthesis.[17,18]

The latter can be explained by gingival healing after surgery, resulting in a space around the pillars. In turn, in the case of a conventional load, impressions are made after the healing period, preventing the appearance of this space.[11]

All these complications are solved by adjusting the prostheses without affecting the results of the procedures. All the variables mentioned above are of high importance to reduce the risk of peri-implantitis.[13]

In view of the above, there is a need to clinically evaluate the implant survival rate in patients with total rehabilitation via implants that underwent immediate loading in the last 5 years.

Material and methods

Study characteristics

This was a cross-sectional, descriptive, observational study based on the completion of a questionnaire, carried out by a single dentist calibrated and approved by the ethics committee of the University Institute of Health Sciences

(Instituto Universitário de Ciências da Saúde – IUCS) in Gandra, Portugal, with the aim of analyzing the osteointegration of the implants loaded immediately on toothless jaws.

Sample characteristics

The target population consisted of patients with edentulous jaws rehabilitated with fixed implant-supported prostheses.

Data collection

A questionnaire was developed. It was administered by a single dentist to avoid the calibration of several stakeholders, between November 2018 and March 2019, with the patients' clinical and formal consent.

The questionnaire was divided into 4 sections:

– participants' personal data – sociodemographic data was acquired for patient characterization; the section included details such as gender, age, risk factors, and the reason(s) for tooth loss;
– characteristics of the rehabilitated area – the obtained information allowed identifying which jaw was rehabilitated and detecting the presence or absence of bone defects;
– surgical characteristics – the parameters covered in this part allowed for the description of factors that were most relevant for treatment in the present study (whether there was a need to perform grafts, the technique used, and the length, diameter and brand of the implants); the osteointegration of the implants was also addressed, identifying how many of them were lost;
– complications – in this section, it was checked whether there were any complications, what they were and when they occurred.

Inclusion and exclusion criteria

The analysis embraced edentulous patients rehabilitated by means of fixed implant-supported prostheses with immediate loading, with a follow-up of up to 5 years.

All patients with incomplete or non-existent clinical information were excluded from the questionnaire.

Statistical analysis

The data obtained in the questionnaire was grouped in MS Excel® (Microsoft Corporation, Redmond, USA) before proceeding to the statistical analysis. All the results were statistically analyzed using IBM SPSS Statistics for Windows, v. 24.0 (IBM Corp., Armonk, USA). Descriptive statistics, appropriate for each variable, were used as well as the analysis of absolute and relative frequencies. The questionnaire referred to qualitative variables; accordingly, the χ^2 test of independence was used. In all the tests, a significance level (α) of 5% was used, i.e., $\alpha = 0.05$. There are some requirements regarding the χ^2 test, without which it cannot be applied. It is assumed that the expected frequencies are higher than 1 and that at most 20% of them are lower than 5. This test presents as a null hypothesis (H0) that the variables are independent; as an alternative hypothesis (H1), the variables are not independent, thus they are related. There is statistical evidence showing that the variables are related when the p-value associated with the test is below the determined significance level, i.e., when the p-value is <0.05. In cases where the χ^2 test of independence could not be applied, classes were grouped so that the test verified the respective assumptions.

Results

To perform this study, a questionnaire was administered. Therefore, this section presents its results.

Sample description

Of a total of 103 participants, 50 (48.5%) were female and 53 (52.5%) were male. Thus, there was a homogeneous distribution of both genders.

Regarding the age distribution (Table 1), the group aged 56–65 years was the largest in the sample with a percentage of 54.4% (n = 56), followed by the group aged 46–55 with a corresponding percentage of 21.4% (n = 22) and the group of participants over 65 years old with a percentage of 19.4% (n = 20). Only 5 persons (4.9%) were under 45 years of age.

Table 1. Distribution by participant age

Age [years]	Frequency n	Valid percentage [%]	Aggregate percentage [%]
≤35	1	1.0	1.0
36–45	4	3.9	4.9
46–55	22	21.4	26.3
56–65	56	54.4	80.6
>65	20	19.4	100.0
Total	103	100.0	–

Regarding the risk factors, more than half of the sample declared to be healthy with a matching lifestyle. Thus, 64.1% (n = 66) did not declare any of the risk factors. Only 19.4% (n = 20) were diabetic. Twelve participants (11.7%) were smokers. A very small percentage of the participants (4.9%) had bruxism (Table 2).

As to the reasons for tooth loss, the most common answers were periodontitis (40.8%) and caries + periodontitis (41.7%). Only 5 persons (4.9%) reported having lost teeth due to trauma and 13 others (12.6%) referred to caries as the main cause.

Of the total sample, 58.3% (n = 60) rehabilitated the maxilla and 41.7% (n = 43) rehabilitated the mandible.

Table 2. Distribution by risk factors

Risk factor	Frequency n	Valid percentage [%]	Aggregate percentage [%]
Bruxism	5	4.9	4.9
Smoking habit	12	11.7	16.6
Diabetes	20	19.4	35.9
None	66	64.1	100.0
Total	103	100.0	–

The dentists responsible for maxillary rehabilitation were questioned about the possible interference of a bone deformity in the process. The vast majority of the patients did not present any bone deformities, corresponding to 91.3% (n = 94) of the sample.

Oral rehabilitation data

The dentists were asked about some aspects of the rehabilitation they had performed. The vast majority did not find it necessary to perform bone grafts prior to implant placement.

Accomplishment

The dentists were asked about the success of rehabilitation. Approximately 70.9% (n = 73) reported successful procedures and 29.1% (n = 30) admitted treatment failure.

For those who were successful, the questionnaire ended here.

Failure

The authors of the present study wished to learn what caused failed cases.

Four out of the 30 previously presented unsuccessful cases did not include information about what had caused the failure. Therefore, they were excluded from the analysis of this item.

The failure with the highest incidence, with 46.2% (n = 12) of the answers, was the fracture of the prosthetic part. The second most recurrent cause, but with a significantly lower percentage, was peri-implantitis, comprising 23.1% (n = 6) of the answers, followed by secondary stability (15.4%) and unscrewing (11.5%). Primary stability failed in only 1 (3.8%) of the studied cases.

We also wanted to know the length of time between rehabilitation and failure. The most frequent answer (88.5%) indicated that failure occurred 6 months after rehabilitation. In only 3 cases of rehabilitation, failure occurred in a period of time shorter than or equal to 6 months.

In 91 out of the 103 study cases, no implants were lost. However, we wanted to know how many failed. Among 12 rehabilitation cases in which implants failed, there were 2 reported cases of 3 lost implants (16.7%). In the remaining cases there was 1 failed implant (83.3%).

Variable cross-checking

To verify whether there were any significant relationships between the variables, we used the χ^2 test of independence.

In this test, the null hypothesis (H0) assumes that there is no association between the variables – they are independent; therefore, this hypothesis must be rejected.

It is concluded that the variables may be associated if the resulting p-value is under 5%. However, the use of this test depends on the validation of 2 assumptions – that a maximum of 20% of the cross-table cells have the expected values lower than 5 and that the expected minimum value is greater than 1.

Relationship between maxillary and mandibular rehabilitation and success

Of the 60 persons with implants in the upper jaw, 40 (66.7%) were successful. That success percentage rose to 76.7% for mandibular rehabilitation.

The resulting p-value was 0.267 (>0.05). Therefore, the type of rehabilitation and the rate of success were not significantly related issues (Table 3).

Table 3. Distribution according to the relationship between maxillary and mandibular rehabilitation and success

Jaw	Success				Total	
	yes		no			
	n (%)	success rate [%]	n (%)	success rate [%]	n (%)	success rate [%]
Maxilla	40 (66.7)	54.8	20 (33.3)	66.7	60 (100.0)	58.3
Mandible	33 (76.7)	45.2	10 (23.3)	33.3	43 (100.0)	41.7
Total	73 (70.9)	100.0	30 (29.1)	100.0	103 (100.0)	100.0

Relationship between the risk factors and success

In this case, the χ^2 test could not be applied; thus, bruxism and smoking habits were grouped. The results of the new data crossing are presented below (Table 4).

Of the 17 smokers with bruxism, 13 (76.5%) had successful rehabilitation and 4 (23.5%) did not.

Of the 20 diabetic persons, 18 (90%) had successful rehabilitation.

Of the 66 persons with no risk factors, 42 (63.6%) were successfully rehabilitated and 24 (36.4%) were not.

The resulting p-value was 0.065 (>0.05). Therefore, the risk factors and the rate of success were not significantly related (Table 5).

Table 4. Distribution of the correlation between the risk factors and the success rate

Risk factor	Success				Total	
	yes		no			
	n (%)	success rate [%]	*n* (%)	success rate [%]	*n* (%)	success rate [%]
Bruxism	3 (60.0)	4.1	2 (40.0)	6.7	5 (100.0)	4.9
Smoking habit	10 (83.3)	13.7	2 (16.7)	6.7	12 (100.0)	11.7
Diabetes	18 (90.0)	24.7	2 (10.0)	6.7	20 (100.0)	19.4
None	42 (63.6)	57.5	24 (36.4)	80.0	66 (100.0)	64.1
Total	73 (70.9)	100.0	30 (29.1)	100.0	103 (100.0)	100.0

Table 5. Distribution according to the relationship between the risk factors and rehabilitation success with bruxism and smoking groups

Risk factor	Success				Total	
	yes		no			
	n (%)	success rate [%]	*n* (%)	success rate [%]	*n* (%)	success rate [%]
Bruxism/ Smoking habit	13 (76.5)	17.8	4 (23.5)	13.3	17 (100.0)	16.5
Diabetes	18 (90.0)	24.7	2 (10.0)	6.7	20 (100.0)	19.4
None	42 (63.6)	57.5	24 (36.4)	80.0	66 (100.0)	64.1
Total	73 (70.9)	100.0	30 (29.1)	100.0	103 (100.0)	100.0

Relationship between the technique used and the success rate

In the All-on-4 technique, of the 68 cases, 48 were successful and 20 were not. In the All-on-5 technique, 5 (62.5%) of the 8 cases were successful and 3 (37.5%) were not. Finally, in the All-on-6 technique, 74.1% of cases were successful.

Among the successful cases, the most commonly used technique was All-on-4 (65.8%), followed by All-on-6 (27.4%), and finally All-on-5 (6.8%).

The resulting p-value was 0.815 (>0.05). Therefore, the technique used and the success rate were not significantly related (Table 6).

Implant data

The applied techniques were classified according to the number of implants used for maxillary and mandibular rehabilitation. A total of 66.0% (n = 68) implants underwent the All-on-4 procedure, while 26.2% (n = 27) underwent the All-on-6 procedure and a minority – 7.8% (n = 8) – utilized the All-on-5 procedure.

Table 6. Distribution of the correlation between the technique used and the success rate

Technique	Success				Total	
	yes		no			
	n (%)	success rate [%]	*n* (%)	success rate [%]	*n* (%)	success rate [%]
All-on-4	48 (70.6)	65.8	20 (29.4)	66.7	68 (100.0)	66.0
All-on-5	5 (62.5)	6.8	3 (37.5)	10.0	8 (100.0)	7.8
All-on-6	20 (74.1)	27.4	7 (25.9)	23.3	27 (100.0)	26.2
Total	73 (70.9)	100.0	30 (29.1)	100.0	103 (100.0)	100.0

Regarding the brand of the implants used, in 46.6% of cases (n = 48), Nobel was chosen. Mis and Strauman were chosen by a minority of the inquired dentists – only 8.7% (n = 9). MegaGen was the choice of 26 dentists (25.2%) (Table 7).

Table 7. Distribution according to the implant brand

Brand	Frequency *n*	Valid percentage [%]	Aggregate percentage [%]
Nobel	48	46.6	46.6
Mis	7	6.8	53.4
Strauman	2	1.9	55.3
MegaGen	26	25.2	80.6
Other	20	19.4	100.0
Total	103	100.0	–

The length and diameter of the implants used also varied.

Most of the surveyed dentists – 83.5% (n = 86) – opted for implants of a length between 10 and 13 mm. The options 'less than 10 mm' – 1.9% (n = 2) – and 'more than 13 mm' – 14.6% (n = 15) – were not frequently chosen.

Most dentists – 77.7% (n = 80) – used implants whose diameter varied between 3 and 4 mm. Only 22.3% (n = 23) opted for diameters greater than 4 mm.

Osteointegration

Then, the binomial test was performed to assess the proportion of osteointegration. The null hypothesis to be tested was that the non-osteointegration ratio was 3%, i.e., the osteointegration rate was 97%. The test yielded a p-value of 0.351 (>0.05). At a significance level of 5%, it could not be concluded that non-osteointegration was greater than 3% and osteointegration was not less than 97%.

Differences in osteointegration between the jaws

To verify whether there were significant differences in osteointegration between the jaws, the *t* test was used. This test led to a *p*-value of 0.443 (>0.05). Therefore, despite the differences observed in the previous description, these differences were not significant. That is, there were no significant differences in osteointegration between the 2 jaws. The χ^2 test led to a *p*-value of 0.442 (>0.05), so the variables were not related, and the jaw and osteointegration were independent of one another (Table 8).

Table 8. Distribution of upper and lower jaw osteointegration (total number of implants $N = 474$)

Osteointegration	Upper jaw *n* (%)	Lower jaw *n* (%)
Yes	273 (97.2)	185 (95.9)
No	8 (2.8)	8 (4.1)
Total	281 (100.0)	193 (100.0)

Discussion

The present study comprised a sample of 474 implants placed in 103 patients. It can be considered a very satisfactory sample size, since the work in question can be categorized as a cross-sectional, observational study, and similar studies can be found in the literature that have an identical or lower sample size in comparison with the present one. The traditional method of distribution of the questionnaire (filling in paper) proved to be ideal for obtaining a greater number of responses. The questionnaires were completed by the author in the presence of patients, providing greater answer reliability. The choice of the region for the application of the questionnaire (Porto, Portugal) was conditioned by the researcher's easier access and communication to the potential participants in this particular area.

Due to the use of the traditional method for disseminating the questionnaire, data processing and the statistical analysis required a greater amount of time on the part of the researcher, since it was necessary to manually enter all the responses/results into the computer program. This manual process of filling in/inserting data can lead to an error associated with manual transposition. The main objective of the present investigation was to evaluate the success rate of immediate-load rehabilitation in edentulous jaws. This assessment was carried out based on the patients' responses to the questionnaire and the review of clinical records, so the existence of a response bias should be considered. This may be particularly evident in questions regarding the reason(s) for tooth loss and the presence of risk factors, where participants may have been influenced to respond according to what is most accepted or considered most correct. It is known that to use an immediate loading protocol, certain patient selection criteria must be followed.[18] It was found that the presented sample was mostly healthy; however, we had 19.4% diabetics, 11.4% smokers and 4.9% bruxism patients. When these variables were correlated, it was found that the risk factors and the occurrence of complications were not significantly related. The primary reason for tooth loss was periodontitis (82.5%), with the remainder divided between caries and trauma. More maxillae than mandible jaws were rehabilitated (58.3% and 41.7%, respectively). The vast majority (91.3%) did not present bone defects and only 12.6% required grafts. The most commonly used technique was All-on-4, which can be explained by the lower number of implants the technique requires; therefore, it becomes more economical and easier to sanitize the hybrid denture. The All-on-6 technique was the second choice with 26.2%, and finally the All-on-5 technique with 7.8%. We found that the technique used and rehabilitation success were not statistically significantly related, emphasizing what was mentioned in 2005 by Balshi et al., who claimed to find no relationship between the number of implants and success.[19] However, Szmukler-Moncler et al. claimed that 6 implants was the appropriate number to achieve a predictable result.[15] For Maló et al.,[10,20] the All-on-4 technique provided very predictable and successful results.[15] The implants were placed in both jaws. Since more maxillae than mandibles were rehabilitated, depending on the number of implants used in each technique, it was found that more implants were placed in the upper jaw. The implant length chosen in this study was mostly between 10 and 13 mm (83.5%). These values are within what is considered an implant length (≥10 mm) adequate to predictably perform total rehabilitation via an immediate load. However, Balshi et al. say they prefer to use longer implants, whenever possible, stating that the lengths between 13 and 15 mm are frequently used.[19,21] In situations of low bone height, the inclination of implants may be a solution for posterior regions.[19] Regarding the diameter, Balshi et al. state that 4-millimeter-wide implants are generally the first choice.[21] Accordingly, in this study, the diameter oscillated between 3 and 4 mm, making up 77.7% of the implants placed. Only 22.3% of the implants placed had a diameter greater than 4 mm. Maló et al.[20] and Javed et al.[14] reported higher failure rates in implants of larger diameters, which was associated with implantation in bone types III and IV.[21]

The existence of some complications was verified in this study. The most frequent one was the fracture of the prosthesis (46.2%), followed by peri-implantitis (23.1%), and finally unscrewing (11.5%). Most cases occurred 6 months after surgery (88.5%). Only 11.5% of cases occurred in the first 6 months after surgery.

We found that the type of jaw and the occurrence of complications were not statistically significantly related.

Both biological and prosthetic complications are rare in studies of immediate loading with complete-arch prostheses. Pain and edema are common.[19]

According to Gallucci et al.,[22] the survival rate of the temporary prostheses used in their study, in immediate loading, was 100%.[23] Balshi et al. reported a rate of 99.0%.[19] On the other hand, Jaffin et al. replaced a fixed temporary prosthesis with a removable prosthesis during osteointegration due to the loss of several implants.[24]

In this study, of the 474 implants placed, 16 did not osteointegrate, with an osteointegration rate of 97%. We found that there were no significant differences in osteointegration between the 2 jaws, and that the technique used and osteointegration were independent.

In 2014, Balshi et al. proved a higher success rate of the All-on-4 technique in edentulous jaws as compared to dentulous jaws.[21] However, in 2011, Maló et al. studied the viability of the All-on-4 technique in a medium and long term in edentulous jaws, obtaining an implant survival rate of 98% in a sample of 968 immediate-load implants.[20] They concluded, therefore, that it was a viable technique that worked as a good medium- and long-term treatment alternative.[20] However, in 2015, Lopes et al., in a study with a sample of 92 implants placed according to the All-on-4 concept, obtained survival rates of implants and prostheses of 96.6% and 100%, respectively, at 5 years of follow-up.[25] They concluded, therefore, that the technique was safe and predictable.[25] According to Gallucci et al.,[22] 2 articles published by Grunder[26] and Cooper et al.[27] reported a survival rate of 97 implants placed in 15 patients, which ranged from 97.7% to 100%. One of the articles reported non-prosthetic failure, with the prosthesis survival rate being 100%.[22]

In a prospective study, Balshi et al. suggested that an immediate loading protocol was suitable for all types of patients who needed oral rehabilitation.[19] Tarnow et al. immediately loaded 36 implants and obtained a 97.4% survival rate.[28]

Jaffin et al. reported a 93% osteointegration rate in 236 implants placed to rehabilitate 34 patients.[24] The main causes of failure were identified as an inadequate prosthesis and the chewing of hard foods, which resulted in the micromotions of dental implants, interferring with the process of osteointegration.[24]

Conclusions

Within the limitations of this study and considering the proposed objectives, we can conclude the following:

The rate of osteointegration of the 474 implants placed with an immediate loading protocol was 97%. This rate is in agreement with the rate described in the literature. This outcome indicates that this is a predictable alternative to a conventional cargo.

It was found that the type of jaw and the occurrence of complications were not related. Therefore, the rehabilitation of the mandible or the maxilla will not necessarily bring associated complications.

The risk factors and the occurrence of complications were also unrelated. In other words, it was possible to successfully rehabilitate patients with risk factors, just as the healthy ones.

The rate of osteointegration did not vary depending on the rehabilitated jaw. These variables were independent.

The technique used and osteointegration proved to be independent as well.

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Original papers

Influence of the dental implant number and load direction on stress distribution in a 3-unit implant-supported fixed dental prosthesis

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Abstract

Background. The choice between 2 or 3 implants to support a 3-unit implant-supported fixed dental prosthesis (FDP) still generates doubt in clinical practice.

Objectives. The aim of this study was to evaluate stress distribution in 3-unit implant-supported FDPs according to the implant number and load direction.

Material and methods. A numerical simulation was performed to analyze stress and strain according to the implant number (2 or 3) and load direction (axial or oblique). A model of a jaw was created by means of the modeling software Rhinoceros, v. 5.0 SR8. External hexagon implants, micro-conical abutments and screws were also modeled. The final geometries were exported to the computer-aided engineering (CAE) software Ansys, v. 17.2, and all materials were considered homogeneous, isotropic and elastic. Different load directions were applied for each model (300 N) at the center of the prosthesis.

Results. The von Mises stress and strain values were obtained for the titanium structures and the bone, respectively. The implant number influenced the prosthesis biomechanics, with higher stress and strain concentrations when 2 implants were simulated. The oblique load also affected the mechanical response, showing higher stress and strain in comparison with the axial load, regardless of the implant number.

Conclusions. It was concluded that for a 3-unit implant-supported FDP, a greater number of implants associated with axial loads can result in a better mechanical response during chewing.

Key words: dental implants, finite element analysis, biomechanics, stress distribution, fixed dentures

Cite as
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Introduction

Currently, implant-supported rehabilitation can be applied as predictable treatment.[1] The literature affirms that implant-supported fixed dental prostheses (FDPs) increase the life quality of edentulous patients.[2] However, some factors can affect the FDP longevity, e.g., the stress concentration, bone quality, or the dimensions and position of the implants.[3]

Considering the biomechanical response of an implant-supported FDP, the implant number is an important factor, since it may have an influence on how the masticatory load will be transmitted to the bone tissue.[4] A large number of implants have a decisive effect on the mechanical response to prosthetic treatment.[5] Placing an implant for each missing tooth is controversial and requires careful consideration in the most complex clinical situations.[6]

For a 3-unit implant-supported FDP, the choice between the use of 2 or 3 implants is related to the masticatory load and bone availability.[7] When 3 implants are used, it is expected that less load will be transferred to the bone–implant interface.[8] When more implants are used, the mechanical response from the central implant can be improved.[9] However, it has been reported that the load direction also can modify the stress concentration in an implant-supported FDP.[10] The biomechanical behavior of abutments, prosthetic screws and dental implants have not been investigated yet by comparing 2 or 3 implants with different load directions.

During chewing, the prosthetic components are constantly subjected to the combination of horizontal, vertical and oblique forces.[11] The axial forces exerted on the implant are compressive in nature; however, horizontal or oblique resultant forces can increase lateral displacement, and consequently cause the formation of torsional forces and lever points, which – when excessive – can lead to failure in the prosthesis structure and at the bone–implant interface.[12] Non-axial loads in dental implant mechanical systems leave the prosthetic components at higher risk of failure due to fatigue.[13]

The number of implants depends on the type of rehabilitation that is to be performed. To improve the stability and support of an overdenture, 2–4 implants are recommended.[14,15] In the literature, one can find studies which show implant survival data ranging from 95% to 100% for cases with 2 and 4 implants, and 81.8–96.1% for 1 and 2 implants with immediate loading.[16,17] A 3-unit FDP can resist a load of 500–600 N in the posterior region, regardless of the load condition.[17] However, for a 3-unit implant-supported FDP, the literature is still scarce.

The finite element analysis (FEA) is an effective methodology for the in silico investigation of the mechanical response of FDPs.[9–11] The finite element analysis can be used to analyze the mechanical behavior, the stress distribution and the direction of forces. When studying the longevity of prosthetic rehabilitation, biomechanics is of great importance in predicting failure in implants, since occlusal overload is a major cause of bone loss around implants, and consequently leads to the loss of prosthetic restorations and implants.[18] Therefore, the aim of the present study was to investigate the biomechanical behavior and stress distribution of FDPs supported by 2 or 3 implants, submitted to different masticatory loads, using FEA. The null hypothesis was that there would be no difference in stress and strain according to the implant number and load direction.

Material and methods

The three-dimensional (3D) models consisted of a section of the maxilla and the following elements: external hexagon implants (4.1 mm in diameter, 9 mm in length), mini-conical abutments (4.0 mm in diameter, 1.5 mm in length), abutment screws, prosthetic screws (Titamax Ti; Neodent, Curitiba, Brazil), and a prosthesis. The models were created using the computer-aided design (CAD) software Rhinoceros, v. 5.0 SR8 (McNeel North America, Seattle, USA). The bone tissue was modeled as well; it contained the cortical bone of a thickness of 1.0 mm and the cancellous bone. The FDPs were created using a database from the Institute of Science and Technology at São Paulo State University (UNESP) in São José dos Campos, Brazil. They contained a maxillary first premolar, second premolar and first molar.[18] The models were duplicated in 2 groups: the 1st group received 2 implants at the ends of the prosthesis; and the 2nd group received 3 implants, 1 for each tooth, according to Fig. 1. All volumetric solids were assumed to have the same number of faces connected to each other.

All the models were exported to the computer-aided engineering (CAE) software Ansys, v. 17.2 (ANSYS Inc., Houston, USA) in the STEP format, and the tetrahedral elements formed the mesh. The properties of each material are summarized in Table 1; the models were considered homogeneous, isotropic and elastic.

Table 1. Mechanical properties of the materials used in the finite element analysis (FEA)

Structure	Elastic modulus [GPa]	Poisson's ratio
Titanium	110	0.3
Zirconia	220	0.3
Cortical bone	14	0.3
Trabecular bone	1.4	0.3

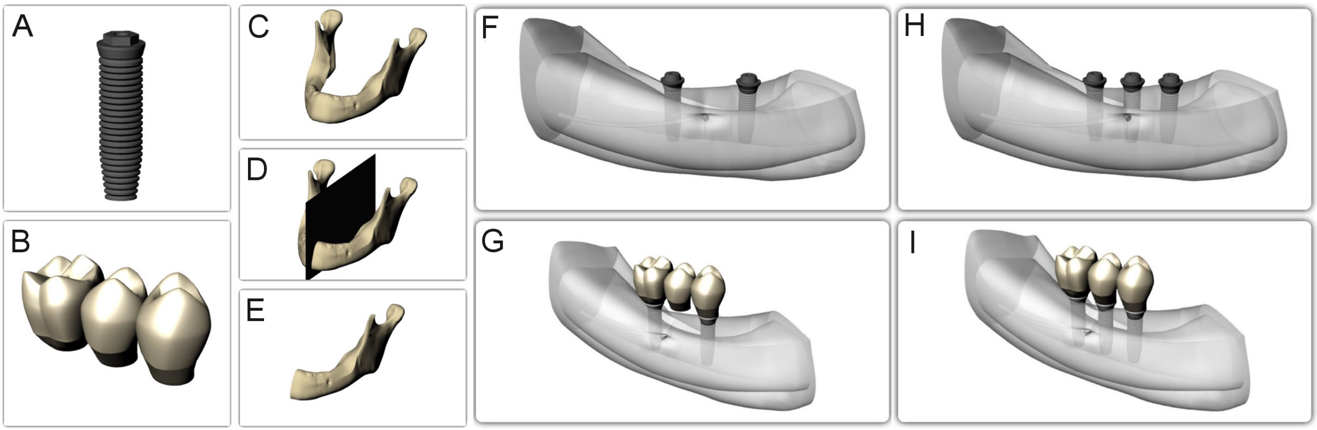

Fig. 1. Three-dimensional (3D) modeling for the numerical simulation

A – external hexagon implant model; B – posterior 3-unit fixed dental prosthesis (FDP); C – edentulous jaw; D – cutting plane for the section model; E – hemi-jaw model; F – 2-implant model; G – 2-implant model with the FDP; H – 3-implant model; I – 3-implant model with the FDP.

The models were restricted in all directions on the cortical bone surface. The zones of non-linear contact were defined in 4 critical interfaces: implant–bone; implant–abutment; implant–screw; and abutment–screw. The contact analysis defined the load transfer and deformation between different components. The friction coefficient was defined as 0.3 for all titanium–titanium interfaces,[19] 0.65 for the cortical bone–implant interface[20] and 0.77 for the cancellous bone–implant interface.[21]

For the masticatory force simulation, a load of 300 N[22,23] was applied to the center of the prosthesis in 2 different directions: axial to the central implant; and obliquely at 45° in relation to the long axis of the central implant.

The results were required as the von Mises stress criteria for ductile solids. For the peri-implant tissue, the required result was expressed as the microstrain value based on a previous study, which correlated this criterion with bone reabsorption.[24] Results that presented a difference in stress peaks between the same regions which was higher than 10% were defined as significant based on the mesh convergence test.[21–23]

Results

Regarding the implant number and load direction, the strain values in the bone were plotted in colorimetric graphs (Fig. 2). It could be observed that the larger the number of implants to support the FDP was, the less strain was concentrated in the bone tissue (Fig. 2A,2C and Fig. 2B,2D). One can notice that strain was homogeneously concentrated in the cervical region between all the implants. The oblique load increased the strain magnitude, regardless of the implant number (Fig. 2B,2D).

With regard to von Mises stress in the dental implants, it could be observed that the external hexagon region was the most involved in the stress concentration, regardless of the model (Fig. 3). However, using the central implant may reduce the stress magnitude, even for the oblique load. The same behavior can be noted for the microconical abutment (Fig. 4) and the prosthetic screw (Fig. 5).

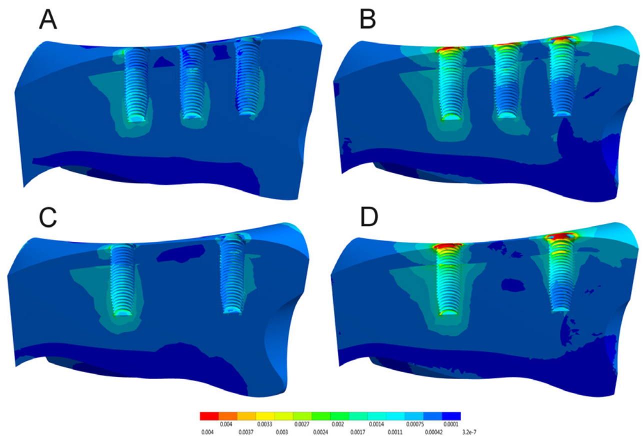

Fig. 2. Bone microstrain maps for 3 implants with the axial load (A), 3 implants with the oblique load (B), 2 implants with the axial load (C), and 2 implants with the oblique load (D)

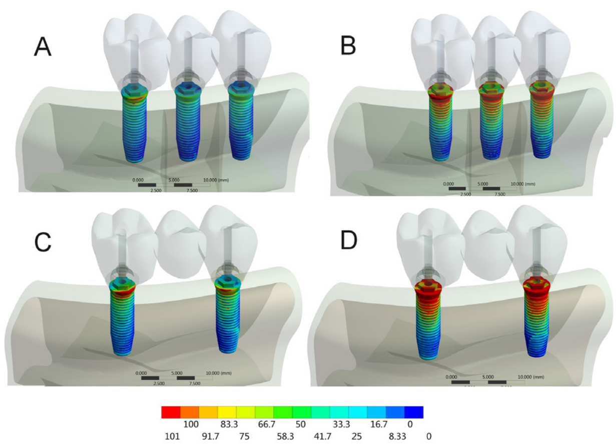

Fig. 3. von Mises stress maps in the implants for 3 implants with the axial load (A), 3 implants with the oblique load (B), 2 implants with the axial load (C), and 2 implants with the oblique load (D)

The microstrain peaks and stress peaks were recorded for each tooth and are summarized in Table 2.

According to Wolff's law,[24] strain values below 50 mm/mm are able to promote bone remodeling due to disuse, and values above 3,000 mm/mm are able to promote bone remodeling by microdamage. There was no simulated model which could prevent or promote bone remodeling; however, the use of 3 implants with the axial load may reduce the risk of bone remodeling.

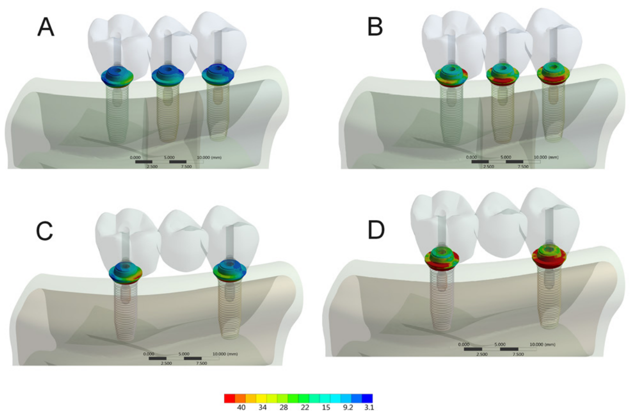

Fig. 4. von Mises stress maps in the micro-conical abutment for 3 implants with the axial load (A), 3 implants with the oblique load (B), 2 implants with the axial load (C), and 2 implants with the oblique load (D)

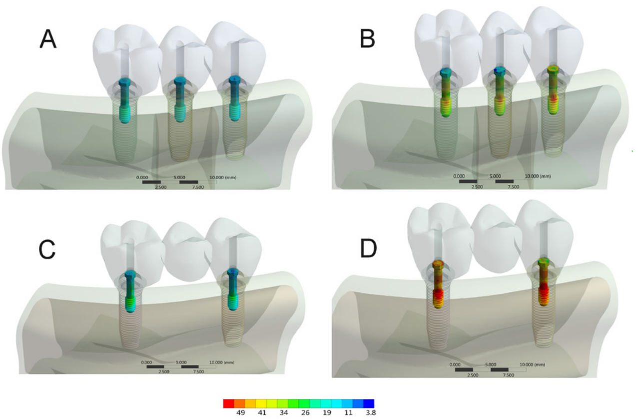

Fig. 5. von Mises stress maps in the prosthetic screw for 3 implants with the axial load (A), 3 implants with the oblique load (B), 2 implants with the axial load (C), and 2 implants with the oblique load (D)

Discussion

This study evaluated the influence of the implant number and load direction in an implant-supported FDP on the bone strain and von Mises stress. The results showed that there was a direct relationship between the implant number and the calculated strain. It was also possible to observe that the axial load promoted less stress and strain. Based on the results, the null hypothesis was rejected, because there was an influence of the load and implant number on the mechanical response.

A previous study evaluated the influence of the number of implants on the biomechanical behavior of overdentures, using the FEA method.[25] In overdentures retained by 2 implants, the level of stress in the peri-implant bone and on the abutment was higher in comparison with a situation when 3 implants were used. According to the authors, the stress generated around the central implant was reduced, which was beneficial for the peri-implant bone.[25] The present study corroborates this statement, since the use of 3 implants reduced the risk of bone remodeling and damage to the abutments, screws and implants.

Another investigation evaluated the effect of the diameter, length and number of implants on stress distribution in the peri-implant bone of a 3-unit implant-supported FDP.[26] The authors concluded that the use of only 2 implants of a diameter of 4.1 mm was sufficient to retain partial prostheses without causing damage to the cortical bone. In addition, the researchers reported that when using the central implant in the case of the prostheses supported by 3 implants, the magnitude of the generated stress could be reduced, which did not occur in the case of the prostheses supported by only 2 implants.[26] The present study corroborates those observations, since the use of the central implant in the case of the prosthesis supported by 3 implants reduced the magnitude of stress, even for the oblique load. In addition, the present study complements this previous report, showing that the micro-conical abutment and also the prosthetic screw may present a reduced stress concentration when 3 implants are used.

Table 2. Results in terms of bone microstrain [με] and stress [MPa] peak values according to the load direction and implant number

Region	Axial load						Oblique load					
	2 implants			3 implants			2 implants			3 implants		
	molar	first premolar	second premolar	molar	first premolar	second premolar	molar	first premolar	second premolar	molar	first premolar	second premolar
Bone [με]	2,202	215	1,954	1,916	641	544	4,189	278	3,967	2,991	2,789	2,836
Implant [MPa]	21.5	–	21.3	18.3	11.8	4.9	66.6	–	61.7	30.3	30.4	28.8
Micro-conical abutment [MPa]	31.7	–	24.8	25.5	15.5	14.7	40.2	–	44.3	37.8	36.8	38.1
Prosthetic screw [MPa]	24.3	–	24.7	16.7	15.4	15.3	64.5	–	55.9	39.6	44.3	45.1

The literature shows that the larger the number of implants is, the less stress is generated in the peri-implant bone.[27,28] In the present study, the results suggest that the use of 3 implants may help reduce the possible strain in the cortical bone, thus reducing the risk of causing bone damage and bone remodeling; however, axial loads should be planned.

This study simulated a 300-newton load for the test,[29] with 2 different directions – oblique at 45° in relation to the long axis of the implants and axial. In previous studies, one can find different load magnitude used, yet in similar directions.[30,31]

Araki et al. simulated an occlusal load with different directions.[29] However, their results were similar, since it was possible to verify that the highest stress concentration was located in the cervical area of the cortical bone. In this way, the present study corroborates those results, stating that the cervical region is the most affected bone region.[32]

The present study also showed that the use of the central implant decreased the stress concentration, even for the oblique load. The analysis of previous studies shows that the implant design and different lengths also can affect stress distribution.[10,29–32] This must be considered relevant in the surgical planning, focusing on reducing the stress concentration and the cervical bone resorption.

Another research, a clinical study performed on unitary implants, reported the development of some problems before the catastrophic fracture of an implant, e.g., the loosening of the screw, peri-implantitis and the peri-implant bone resorption.[33] Problems such as the peri-implant bone resorption can be noticed in unit rehabilitation, with crowns of an inadequate height or unsuitable for the patient's occlusal function. Due to the dissipation of axial and oblique loads, the larger the number of implants in rehabilitation, the lower the stress concentration that can generate bone resorption around the implants. In this way, the present study suggests that the load direction should be possibly the most axial to reduce the failure risk.

Over the last decades, the FEA method has become an increasingly useful tool to predict the effects of the generated stress on implants and the peri-implant bone.[34,35] This method allows the analytical assessment of load distribution on the implant structure, controlling the possible microstrain as well as identifying the load direction and understanding the impact of the implant number.[36]

In implantology, FEA has been widely used to study the stress patterns of the implant components and also in the peri-implant bone. The success of FEA depends on precision in the simulation of the implant surface geometry and structure, according to the characteristics of the implant material, the load and support conditions as well as the biomechanical and interface conditions. Since it was an in silico study, clinical conditions might have not been fully replicated.[37,38] The stress analysis is normally performed under static load, and the mechanical properties of the materials are defined as isotropic and linearly elastic, although this is not the case in reality.[39] Thus, this analysis allows predicting biomechanical behavior in different situations; still, the results must also be considered qualitatively.

It is important to note that the absence of a microgap in the present model should be considered as a limitation of this study, since it is reported to be a factor that could affect the bone response as well as the implant structure, types of surfaces, shapes, and materials used in the prosthetic system.[40]

In this study, it can be noticed that the use of a greater number of implants reduced the stress concentration under oblique load. However, another way to achieve better load distribution could be the manufacturing of prosthetic crowns that would conform with the patient's occlusal reality, of sizes and shapes adequate to the chewing function. Digital protocol and chairside can assist in achieving the optimal implant placement and crown design.

Conclusions

Considering the limitations of this study, it can be concluded that for a 3-unit implant-supported FDP, a greater number of implants associated with axial loads can result in a better mechanical response during chewing.

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Original papers

Analysis of complications after the removal of 339 third molars

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Abstract

Background. Extractions of third molars constitute about 90% of the scheduled surgical procedures performed by oral surgeons. Wisdom tooth surgery is associated with complications, such as the lingual and inferior alveolar nerve damage, bleeding, tooth/jaw fractures, tooth displacement into the adjacent anatomical spaces, trismus, infections, and other.

Objectives. The aim of the study was to analyze complications after wisdom tooth extraction in patients treated at the Department of Oral Surgery of Jagiellonian University Medical College in Kraków, Poland, in the years 2016–2018.

Material and methods. A retrospective analysis of the medical records of 339 patients treated in the outpatient setting was performed. The inclusion criterion comprised a single extraction of a third molar. The exclusion criteria were multiple extractions, comorbidities and pregnancy. No antibiotic prophylaxis was used. The incidence of post-extraction complications, such as oroantral communication, postoperative hematoma, acute inflammation of the surrounding tissues, trismus, and transient paresthesia in relation to patient gender and age, the developmental stage and location of the removed tooth as well as the type of surgery were studied.

Results. Perioperative complications occurred in 51 (15.0%) cases, and comprised the acute inflammation of the surrounding tissues in 31 patients, trismus after the removal of 13 lower third molars, oroantral communication after the extraction of 5 upper wisdom teeth, and hematoma as well as a transient sensory alteration of the lingual nerve in 1 case each. Complications were more common in patients who had a surgical extraction of a wisdom tooth with root separation and in cases of lower third molar extractions. No statistically significant correlation was found between the patients' age or gender, the developmental stage of the extracted tooth and the number of observed complications.

Conclusions. Lower third molars and the necessity of surgical extraction with root separation are risk factors for postoperative complications in patients who require wisdom tooth removal. Complications after the removal of third molars are most often inflammatory.

Key words: molar, third, tooth extraction, risk factors, postoperative complications

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Introduction

Extractions of third molars constitute about 90% of the scheduled surgical procedures performed by oral surgeons, and on average 37% of all procedures performed annually.[1] Among the indications for wisdom tooth removal, one can distinguish therapeutic indications, most often associated with problems with the eruption of impacted third molars, especially in the case of recurrent acute or chronic pericoronitis, orthodontic indications, mainly dental arch changes and anterior crowding, and also non-restorable caries lesions, periodontal diseases, neuralgic ailments, cysts, and tumors, when the tooth is removed along with a pathological lesion. It is often necessary to remove a mandibular wisdom tooth from the fracture line. This procedure, combined with the reduction and osteosynthesis of fractures, is performed under hospital conditions and general anesthesia. Prophylactic extractions of retained third molars are performed for example before prosthetic treatment in order to prevent these teeth from eruption under the denture plate.[2] The main local contraindication for the removal of lower third molars is acute inflammation, often combined with trismus, which prevents proper local anesthesia and extraction, usually surgical.

The location of wisdom teeth in the posterior part of the oral cavity as well as the variability of the anatomy and location of these teeth make the percentage of complications during and after their extraction higher than that observed after the removal of teeth from other groups. Complications associated with wisdom tooth extraction are estimated at about 3–30%.[3–10] They are favored by the vicinity of important anatomical structures, such as the lingual and inferior alveolar nerves, facial and inferior alveolar arteries, masticatory muscles, and anatomical spaces, including pterygomandibular, parapharyngeal, retro- and submandibular spaces, and in the case of upper teeth – the maxillary sinus, and the pterygopalatine and infratemporal fossae. Intraoperative complications associated with the removal of third molars may include damage to the inferior alveolar nerve both during anesthesia and extraction.[11,12] The fracture of a wisdom tooth or damage to the adjacent teeth as well as the fracture or luxation of the mandible or the fracture of maxillary tuberosity are usually associated with the use of excessive force by the surgeon. Injuries to the surrounding soft tissues and vascular damage, leading to bleeding during or after surgery, usually result from careless handling of instruments. Particularly unpleasant complications associated with the removal of third molars include the displacement of the entire tooth or its root into the maxillary sinus or the pterygopalatine fossa in the case of upper teeth, and into the mandibular canal or the soft tissues of the floor of the mouth in the case of lower teeth. The most serious, life-threatening complication during wisdom tooth extraction is tooth aspiration to the upper respiratory tract with the subsequent spasm of the laryngeal muscles.[13–17]

Oroantral communication found after the extraction of upper third molars may result from anatomical reasons; it is important to diagnose and treat it according to the indications.[18] Bleeding from the injured bones or soft tissues can be observed in the early postoperative period, with less frequent bleeding from the inferior alveolar artery. The formation of hematomas in the postoperative period is related to damage to the pterygoid venous plexus after the removal of third upper molars and a tight suture of the wound after the surgical extraction of third lower molars. Hemorrhagic complications often result from the general condition of the patient (arterial hypertension, diabetes) and the medications taken, which affect the condition of the haemostatic system.[8,19] After wisdom tooth surgery, inflammatory complications often develop, e.g., dry socket and inflammation of the submandibular lymph nodes with the subsequent formation of a submandibular abscess. The inflammatory process spreading to the surrounding anatomical spaces may include the parapharyngeal space, the skull base and the mediastinum. In immunocompromised patients, phlegmon or bacterial osteomyelitis may develop. The postoperative course after the removal of third molars is often complicated by transient trismus and reversible sensory alterations of the lingual and inferior alveolar nerves.[12,20–22]

Objectives

The aim of the study was to analyze the complications after wisdom tooth extraction in patients treated at the Department of Oral Surgery of Jagiellonian University Medical College in Kraków, Poland, in the years 2016–2018.

Material and methods

A retrospective analysis of the medical records of patients treated in the outpatient setting within 2 years was performed. The inclusion criterion comprised a single extraction of an upper or lower third molar in a patient, regardless of the stage of tooth development. The exclusion criteria were multiple extractions, general comorbidities and pregnancy in women. The type of procedure included simple extractions, surgical extractions with root separation and surgical extractions with flap formation (with an angular incision). No antibiotic prophylaxis was used. In 339 patients, the incidence of post-extraction complications, such as oroantral communication, postoperative hematoma, acute inflammation of the surrounding tissues, trismus, and transient paresthesia in relation to patient gender and age, the developmental stage and location of the removed tooth as well as the method of extraction were studied.

The statistical analysis was performed using the statistics package PQStat, v. 1.8.0.392 (PQStat Software, Poznań/Plewiska, Poland). Associations between categorical variables were analyzed with the χ^2 test or Fisher's exact test for small samples. The rank correlation coefficient between age and the number of complications was calculated with Kendall's τ test and the χ^2 test (for trend assessment). Results were statistically significant for *p*-values below 0.05.

Results

Extraction of third molars was performed in 178 (52.5%) women and 161 (47.5%) men aged 15–69 years. The average age of patients was 32 years. People under 18 years of age constituted 16.5% of the respondents. Wisdom tooth extraction was most commonly performed in patients aged 18–38 years. Demographic data is presented in Table 1.

Table 1. Demographic data of the patients

Age [years]	Women *n*	Men *n*	Total *n*
15–17	21	35	56
18–38	85	83	168
39–69	72	43	115
Total *n* (%)	178 (52.5)	161 (47.5)	339 (100.0)

In the studied group of patients, the main indication for third molar surgery was recurrent pericoronitis. The following were qualified for extraction: 134 (39.5%) completely erupted teeth; 115 (33.9%) completely impacted teeth; 59 (17.4%) partially impacted teeth; and 31 (9.2%) eighth tooth buds. The lower teeth were removed more often; in own material, it was 178 (52.5%) third molars. Detailed data on the developmental stage and position of the removed third molars is presented in Table 2.

Table 2. Developmental stage and location of the extracted third molars

Stage of development	Upper third molars *n*	Lower third molars *n*	Total *n* (%)
Tooth buds	12	19	31 (9.2)
Partially impacted teeth	21	38	59 (17.4)
Completely impacted teeth	41	74	115 (33.9)
Completely erupted teeth	87	47	134 (39.5)
Total *n* (%)	161 (47.5)	178 (52.5)	339 (100.0)

The method of extraction in 339 patients was analyzed. In 205 (60.5%) cases, surgical extractions with flap formation by means of an angular incision were performed. This procedure was done while removing 131 lower and 74 upper third molars. Root separation was performed during the extraction of 42 lower third molars. Germectomy was performed in 9 girls and 22 boys under 18 years of age, slightly more often removing lower tooth buds. In other cases, simple extractions were performed. The type of procedure depending on the stage of development of the removed third molars is presented in Table 3.

Table 3. Type of surgery depending on the stage of development of the extracted third molars

Stage of development	Type of surgery *n* (%)	
	flap operation	root separation
Tooth buds	31 (15.1)	0 (0.0)
Partially impacted teeth	48 (23.4)	41 (97.6)
Completely impacted teeth	115 (56.1)	0 (0.0)
Completely erupted teeth	11 (5.4)	1 (2.4)
Total *n* (%)	205 (100.0)	42 (100.0)

Perioperative complications were found in 51 (15.0%) cases. The most frequently observed complication after the extraction of third molars was the acute inflammation of the surrounding tissues, which occurred in 31 patients. In these cases, antibiotic therapy was used. Trismus was found after the removal of 13 lower third molars, oroantral communication was diagnosed after the extraction of 5 upper wisdom teeth and hematoma after the extraction of a lower tooth. A transient sensory alteration in the range of innervation by the lingual nerve was observed in 1 case. Detailed data on the frequency of complications depending on the location of the extracted third molars is presented in Table 4. Fisher's exact test gave a *p*-value of 0.0001, indicating a statistically significant correlation between the location of the wisdom tooth in the mandible and an increased number of postoperative complications.

Table 4. Location of the tooth and the types of postoperative complications in the study group

Complications	Upper third molars *n*	Lower third molars *n*	Total *n* (%)	*p*-value
Infection	13	18	31 (60.8)	0.0001*
Trismus	0	13	13 (25.5)	
Oroantral communication	5	0	5 (9.8)	
Hematoma	0	1	1 (1.95)	
Transient paresthesia	0	1	1 (1.95)	
Total *n* (%)	18 (35.3)	33 (64.7)	339 (100.0)	

* statistically significant.

Dependence between the age of the patients treated and the occurrence of complications is presented in Table 5. Kendall's τ rank correlation coefficient did not reveal a significant association between the age of the patients and the number of complications ($\tau = 0.3333$; $p = 0.6015$). Also the trend assessment did not show any significant correlation ($\chi^2 = 0.4027$; $p = 0.5257$).

Table 5. Relationship between patient age and complications in the study group

Age [years]	Complications *n* (%)	*p*-value
15–17	5 (9.8)	0.6015
18–38	35 (68.6)	
39–69	11 (21.6)	
Total *n* (%)	51 (100.0)	

The relationship between the gender of the patients treated and the incidence of postoperative complications was assessed. However, Fisher's exact test did not reveal any statistically significant association ($p = 0.2450$). The results are presented in Table 6.

The influence of the developmental stage of the extracted tooth on the types of postoperative complications was statistically insignificant, as presented in Table 7.

The relationship between the method of extraction and the incidence of complications was analyzed. Complications were significantly more frequent in the patients who underwent a surgical extraction of a wisdom tooth with root separation ($\chi^2 = 53.74$; $p = 0.0013$). The results are shown in Table 8.

Discussion

According to the literature, the frequency of complications associated with the removal of third molars ranges from 3.7% to 30.9%, with the majority of authors analyzing mainly postoperative complications regarding surgical extractions, whereas, in everyday practice, the non-surgical removal of wisdom teeth is a more common procedure.[3–10,21] The percentage of complications observed in our material, 15% (51/339), is within the limits given in other studies.[5,8,15] In the analyzed group of patients, simple extractions were also included.

In our study group, complications occurred mainly after the extraction of lower third molars (64.7%), which is consistent with the observations of other authors.

Table 6. Relationship between patient gender and the types of postoperative complications

Gender	Infection *n*	Trismus *n*	Oroantral communication *n*	Hematoma *n*	Transient paresthesia *n*	Total *n*	*p*-value
Women	17	11	4	1	1	34	0.2450
Men	14	2	1	0	0	17	
Total *n* (%)	31 (60.8)	13 (25.5)	5 (9.8)	1 (1.95)	1 (1.95)	51 (100.0)	

Table 7. Relationship between the developmental stage of the extracted tooth and the types of postoperative complications

Stage of development	Infection *n*	Trismus *n*	Oroantral communication *n*	Hematoma *n*	Transient paresthesia *n*	Total *n*	*p*-value
Tooth buds	0	1	0	0	0	1	0.3724
Partially impacted teeth	8	4	3	0	0	15	
Completely impacted teeth	4	0	1	0	0	5	
Completely erupted teeth	19	8	1	1	1	30	
Total *n* (%)	31 (60.8)	13 (25.5)	5 (9.8)	1 (1.95)	1 (1.95)	51 (100.0)	

Table 8. Method of extraction and complications in the study group

Method of extraction	Number of complications *n*	Percentage of complications [%]	*p*-value
Surgical extraction with flap formation	205	3.9	<0.0013*
Surgical extraction with root separation	42	40.5	
Simple extraction	92	28.3	
Total	339 (100.0) *n* (%)	15.0	

* statistically significant.

According to Sukegawa et al.,[4] the percentage was 1.94%, while in Sayed et al.'s research, it reaches 80.6%.[3] A high rate of postoperative complications after mandibular wisdom tooth removal is connected with the vicinity of large blood vessels and nerves. Moreover, the density of bone as well as the limited visibility in the distally placed operating field should be taken into consideration.[3,4,16,18]

In several reports, patient age exceeding 30 years, female gender, and surgical extraction with significant bone damage and root separation are defined as risk factors for complications after wisdom tooth extraction.[3,9,22–25] Although in the analyzed group, the influence of the age and gender of the patients treated as well as the developmental stage of the tooth on the incidence of complications was not proven, the method of extraction, especially surgical extraction with root separation, was considered to be a risk factor. In extractions with flap formation, the surgical field was wider, and it was possible to either cut off the crown of the tooth or perform a complete extraction after the cautious removal of the surrounding bone. Careful handling of tissues and instruments prevented our patients from such complications as fracture or luxation of the mandible, fracture of maxillary tuberosity or displacement of the extracted tooth into the adjacent tissues.[13,14,16,17]

The most common complications associated with the removal of third molars include inflammatory reactions, estimated at 0.3–26%.[4,25–27] In our material, this percentage was 9.1% (31/339) for all extractions and 60.8% (31/51) for all complications. According to the literature, the risk of wound infection and the development of alveolar osteitis depends on pre- and postoperative oral hygiene, the type of wound closure and previous pericoronal infection.[4,5,7]

The issue of antibiotic prophylaxis in third molar surgery is widely addressed in the literature. Currently, the opinion that patients requiring wisdom tooth surgery do not benefit from routine antibiotic prescription, as expressed by Menon et al.,[26] predominates. However, there are also some studies showing a slight reduction in the incidence of inflammatory complications in patients who were given antibiotics in the perioperative period.[27] In our material, antibiotics were prescribed only in cases of acute inflammatory complications.

Damage to the lingual or inferior alveolar nerve after the removal of lower wisdom teeth is found in about 5.6% of cases[3,11,12,20,28]; in our study, transient lingual nerve dysfunction was observed in 1 case (1.95%). Despite progress in imaging techniques and the availability of cone-beam computed tomography (CBCT), it is not possible to completely prevent nerve damage in wisdom tooth surgery. According to Pourmand et al., such factors as position of the third molar in relation to the mandibular canal, access to piezo surgery as well as influence of the local anesthetic on the degree of sensation disorders should be also taken into consideration.[29]

Complications occur less frequently after the extraction of upper third molars than lower wisdom teeth.[18,29] Oroantral communication is the most common adverse effect.[16] The incidence of oroantral communication varies from 3.8% to 18.7%.[3,4,16,18,29] In this study, oroantral communication was diagnosed in 3.1% (5/161) of all extracted maxillary third molars and constituted 9.8% of all postoperative complications. The prediction of oroantral communication before surgery is essential both for the patient and the surgeon. According to the literature, the superimposition of the wisdom tooth roots on the maxillary sinus floor, shown on orthopantomogram or CBCT, is one of the most important risk factors.[18,29] Retained upper molars, especially with the distal and mesial inclination of the axis, root fractures and the displacement of the tooth present a higher risk of oroantral communication.[3,4,16]

Extractions of third molars are generally more difficult than those of other molars and require certain surgical skills. Delicate handling of soft tissues and bones, maintaining aseptic conditions of the procedure, proper surgical technique and choice of equipment (instruments, light, suction), and the duration of surgery are related to the experience of the surgeon and should diminish the incidence of peri- and postoperative complications. It is noteworthy that while some authors claim a direct correlation between the level of training and the likelihood of complications,[10,17,25,29] others indicate that the experience of the surgeon has no influence on the incidence of adverse effects.[21]

It should be emphasized that the complications in the analyzed material were mild and transient.

Conclusions

Lower third molars and the necessity of surgical extraction with root separation are risk factors for postoperative complications in patients who require wisdom tooth removal. Complications after the removal of third molars are most often inflammatory.

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Original papers

Evaluation of the participation of hyaluronic acid with platelet-rich plasma in the treatment of temporomandibular joint disorders

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Abstract

Background. Temporomandibular joint disorders (TMDs) are widely researched diseases in medical literature. They are associated with many symptoms, such as pain, limited mouth opening and joint sounds, resulting in decreased quality of life for the patient. Both the hyaluronic acid (HA) injection and the platelet-rich plasma (PRP) injection have a remarkable efficacy in the treatment of TMDs.

Objectives. This study aimed to evaluate the participation of HA with PRP in the treatment of TMDs.

Material and methods. The sample consisted of 24 patients with unilateral or bilateral TMDs. They were divided into 2 groups: HA+PRP was used in the test group (12 patients); and HA alone was used in the reference group (12 patients). The injection protocol for both groups was 4 times at 2-week intervals. Pain at mastication, masticatory efficiency, joint sounds, maximum mouth opening (MMO), and functional limitation in the mandibular movement were evaluated at 2 weeks, 1 month, 3 months, and 6 months after the last injection. The outcome variables were the visual analog scale (VAS) evaluations. The Likert-type scale was used to evaluate the functional limitation in the mandibular movement.

Results. The mean age was 30.58 years in the reference group, and 23.92 years in the test group. There was alleviation of symptoms in both groups through the follow-up periods. There were significant differences between the groups regarding pain at mastication, masticatory efficiency, MMO, and functional limitation at the end of the follow-up period ($p < 0.05$).

Conclusions. The study results suggest that the HA and PRP injection provides greater improvement in patients with TMDs as compared to the HA injection alone; this may be due to taking advantage of the properties of both HA and PRP.

Key words: hyaluronic acid, platelet-rich plasma, temporomandibular joint disorders

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Introduction

Temporomandibular joint disorders (TMDs) are an important problem faced by oral and maxillofacial surgeons and patients; TMDs involve the articular capsule and the associated structures, and it may be the cause of orofacial pain.[1]

Pain complaints due to TMDs are the second most common with regard to the human body (about 33% of musculoskeletal pain). Most people aged 20–40 years have at least 1 of the symptoms of TMDs.[2,3]

Temporomandibular joint disorders include disk displacements, with or without reduction, that may be associated with inflammatory changes, such as capsulitis and retrodiscitis, or with degenerative changes, such as temporomandibular joint (TMJ) osteoarthritis and osteoarthrosis.[3] Common symptoms include pain, which is aggravated by the mandibular movement, joints sounds (clicking or crepitus), a decrease in masticatory efficiency, and functional limitation in the mandibular movement.[4] Medical history, clinical examination and radiographic evaluation are essential methods of diagnosing TMDs.[4] Treatment options for TMDs include self-management, physical therapy, pharmacological management, and TMJ surgery in some cases.[5]

The intra-articular injection of various substances, such as hyaluronic acid (HA) and platelet-rich plasma (PRP), is considered as one of the conservative procedures with high efficacy in the treatment of TMDs.[6–11] The use of intra-articular injections of HA (viscosupplementation), which is a high-molecular-weight polysaccharide, provides the lubrication and protection of the joint cartilage.[1,12–16] Sodium hyaluronate is the sodium salt of hyaluronic acid; it is a physiological component of the synovial fluid and it is responsible for the lubrication of TMJ.[1,17,18]

Many studies have indicated the significant efficacy of the HA injection in the treatment of TMDs.[1,6–8,13,16,17] This is due to its special properties that are similar to those of the synovial fluid – viscosity as well as lubricating and damping capacity.[2] Besides, HA performs mechanical (lubricating the articular elements and reducing their wear), biological (blocking different inflammatory mediators) and metabolic (providing the necessary components of the metabolic process for the articular disk and cartilage) functions.[19–21]

Platelet-rich plasma (PRP) is a concentrated autologous solution of platelets that is obtained by the centrifugation of a blood sample. It contains many growth factors (GFs), such as platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF), and 3 blood proteins (fibrin, fibronectin and vitronectin).[22,23]

In recent years, the clinical applications of PRP have been diversified with regard to several therapeutic and cosmetic fields.[24–26] One of these uses refers to the treatment of joint disorders, including TMDs. Platelet-rich plasma has been shown to decrease signs and symptoms of TMDs, and significantly improve the joint function.[11,27,28]

Hyaluronic acid injections bring immediate, but unsustainable results, unlike PRP injections with their gradual effects, increasing in the long term.[28] Numerous research has lauded the participation of HA and PRP in treating knee problems, such as osteoarthritis and traumatic injuries, and has recorded that this treatment combination is better than injecting each of the substances alone.[29–31]

This study aimed to evaluate the efficacy of the application of HA with PRP in the treatment of TMDs.

Material and methods

Study design and sample

A prospective observational study was carried out with a 6-month follow-up period between December 2018 and February 2020 at the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Damascus University, Syria. The sample included patients referred for the evaluation and management of TMDs. The ethics committee consent was obtained (EC ref. No. 4887, 28/11/2018), and all participants signed an informed consent agreement for inclusion in the study sample.

The sample was randomly divided into 2 groups:
- group I (reference group): 12 patients who received 4 intra-articular injection sessions – 1 mL of HA per session with a 14-day interval between sessions;
- group II (test group): 12 patients treated with an injection of 0.5 mL of HA in combination with 0.5 mL of PRP into TMJ per session for 4 sessions with a 14-day interval between sessions.

A specific information form was filled in for each patient. The information from medical history, clinical evaluation (Fig. 1) and radiographic examination (Fig. 2) was recorded. The patients were evaluated with the Wilkes classification.[32]

The patients in both groups had a diagnosis of intermediate (III), late intermediate (IV) or late (V) stage. The inclusion criteria were as follows: patients suffering from unilateral or bilateral TMDs. The exclusion criteria were patients with systemic diseases (e.g., rheumatoid arthritis, psoriatic arthritis or juvenile arthritis), those receiving therapy with anticoagulants and those who had shown symptoms of hypersensitivity to the HA solution.

Fig. 1. Mandibular movements during clinical evaluation

Fig. 2. Radiographic examination

A – anterior disk displacement (magnetic resonance imaging – MRI); B – temporomandibular joint (TMJ) osteoarthritis (cone-beam computed tomography – CBCT).

Materials

The following materials were used: 2-milliliter syringes of HA (Hyalgan®; Fidia Farmaceutici, Abano Terme, Italy), different sizes of syringes, laboratory tubes containing sodium citrate, dry tubes, a centrifuge, a camera (Sony DSC-W710/B 16 MP; Sony, Tokyo, Japan), and a caliper.

Treatment protocol and injection technique

Group I (HA alone):

After receiving the patient, recording medical history, filling in the patient's form, and completing the diagnostic procedures (clinical, simple radiography, computed tomography (CT) and magnetic resonance imaging (MRI)), the patient was prepared for the 1st injection session, which was conducted as follows:

– an allergy test for HA was performed;
– the lateral canthus–tragus reference points were used (Fig. 3)[33]; the entry point was 10 mm away from the middle of the tragus and 2 mm below the line, the mandibular condyle was forced into the anterior position, with the index finger remaining on the condyle as a safety guide;
– the skin surface was disinfected with povidone-iodine;
– 1 mL of HA was injected into the upper joint space with a 19-gauge needle; after removing the needle, the patient was asked to close their mouth slowly;
– special instructions were given after injection.

The injection was repeated 4 times, with 14-day intervals between sessions.

Fig. 3. Technique used for injections into the temporomandibular joint (TMJ)

Group II (HA+PRP):

The patient was informed not to take antibiotics for a week before and after the injection because of their negative effects on PRP.

Preparation of platelet-rich plasma

The Marx protocol was adopted based on the study by Sabarish et al.,[34] which was preceded by two-step centrifugation procedures. Firstly, under strictly sterile conditions, 10 mL of peripheral blood was collected from the ulnar vein of the patient with a single-use syringe. It was placed in a tube containing sodium citrate as an anticoagulant (1/9), and then it was centrifuged for 4 min at a speed of 1,000 rpm. In the 2nd step, PRP was separated from platelet-poor plasma (PPP) in a dry tube through centrifugation for 9 min at 800 rpm.

As in group I, we followed the same procedure before injection.

Then, 0.5 mL of HA was injected into the upper joint space, which was immediately followed by an injection of 0.5 mL of PRP.

The patient was asked to close their mouth slowly.

The injection was repeated 4 times, with 14-day intervals between sessions.

In an attempt to achieve a single-blind design, we did not inform the patient which of the treatment protocols they received.

Outcome variables

Pain at mastication was assessed using the visual analog scale (VAS) from 0 to 10; 0 meant 'no pain' and 10 – 'the worst pain the patient has ever experienced'.

Mastication efficiency was assessed with VAS from 0 to 10, the extremes of which were 'eating only semi-liquids' and 'eating solid, hard food', respectively.

Joint sounds: the scale was anchored with 'no noise' (score 0) and 'noise as excessive as it could be' (score 10).

Maximum mouth opening was measured in millimeters.

Functional limitation in the mandibular movement was assessed with the Likert-type scale (0 – absent; 1 – slight; 2 – moderate; 3 – intense; and 4 – severe).

All outcome variables were evaluated at 2 weeks, 1 month, 3 months, and 6 months after the last injection.

Statistical analysis

The results were analyzed with the IBM SPSS Statistics for Windows software, v. 24.0 (IBM Corp., Armonk, USA), The Wilcoxon and Mann–Whitney *U* tests were used for intragroup and intergroup comparisons, respectively. Significance was set at a *p*-value less than 0.05 for all statistical tests.

To achieve a statistical study and to check changes within each group, the arithmetic means of each variable were analyzed separately during the follow-up points in both groups, and finally we verified differences between the 2 groups before the treatment and after a 6-month follow-up.

Table 1. Intragroup comparisons of the outcome variables over time

Variable	Groups	Before treatment	*p*-value	2 weeks	*p*-value	1 month	*p*-value	3 months	*p*-value	6 months
Pain at mastication [VAS]	HA	6.500 ±1.648	0.002*	4.479 ±1.568	0.002*	3.313 ±1.716	0.118	2.750 ±1.215	0.026*	3.500 ±1.348
	HA+PRP	6.750 ±2.463	0.002*	3.250 ±2.017	0.037*	2.583 ±1.881	0.363	2.375 ±2.090	0.618	2.250 ±2.105
Mastication efficiency [VAS]	HA	4.708 ±1.824	0.002*	6.417 ±1.276	0.003*	7.458 ±1.389	0.305	7.750 ±1.055	0.040*	7.333 ±1.094
	HA+PRP	3.833 ±2.188	0.002*	7.229 ±1.042	0.023*	7.708 ±1.033	0.234	7.875 ±1.264	0.129	7.897 ±1.435
Joint sounds [VAS]	HA	5.896 ±1.704	0.002*	3.729 ±0.997	0.004*	2.875 ±0.856	0.719	3.000 ±1.022	0.012*	3.958 ±0.964
	HA+PRP	6.917 ±2.076	0.003*	4.333 ±1.600	0.003*	3.042 ±1.437	0.394	2.833 ±2.004	0.340	2.667 ±1.958
MMO [mm]	HA	35.083 ±10.544	0.003*	40.875 ±7.139	0.141	41.750 ±6.088	0.011*	39.667 ±5.047	0.506	39.083 ±6.313
	HA+PRP	34.708 ±9.418	0.004*	43.583 ±5.518	0.893	43.917 ±6.775	0.178	43.042 ±7.257	0.843	42.875 ±7.155
Functional limitation [Likert-type scale]	HA	2.042 ±0.964	0.007*	0.875 ±0.433	0.025*	0.458 ±0.498	0.084	0.708 ±0.620	0.014*	1.083 ±0.515
	HA+PRP	2.458 ±0.891	0.003*	0.875 ±0.644	0.015*	0.458 ±0.582	0.257	0.333 ±0.492	0.180	0.458 ±0.542

Groups: HA – hyaluronic acid alone; HA+PRP – hyaluronic acid with platelet-rich plasma; VAS – visual analog scale; MMO – maximum mouth opening;
* statistically significant.
Data presented as mean (*M*) ± standard deviation (*SD*).

Results

The study sample consisted of 24 patients suffering from unilateral or bilateral TMDs; the sample was divided into 2 groups (HA group and HA+PRP group) with a mean age of 30.58 and 23.92 years, respectively.

The search variables were studied within each group over time by comparing them at each follow-up point with their predecessors, and in general, the results showed significant improvement in the 2 groups in all the variables (Table 1). The particular results were as follows:

Pain at mastication

In both groups, the pain decreased significantly through all follow-up points as compared to the pre-treatment score, with statistically significant differences between the periods before treatment–after 2 weeks and 2 weeks–1 month ($p < 0.05$). In the HA group, the pain returned to increase with a statistically significant difference between the periods 6 months–3 months, in contrast to the HA+PRP group, where it continued to decline until the end of the follow-up (Fig. 4).

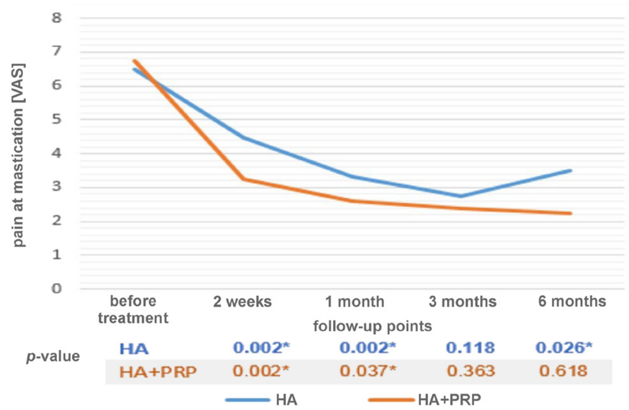

Fig. 4. Pain at mastication at the time of the study

* statistically significant.

Mastication efficiency

For this variable, an increase was expected; differences between the means were statistically significant when comparing the periods before treatment–after 2 weeks and 2 weeks–1 month ($p < 0.05$) in both groups.

The mastication efficiency increase in the HA+PRP group continued until the end of the follow-up, while it decreased in the HA group, with a statistically significant difference from the previous period (Fig. 5).

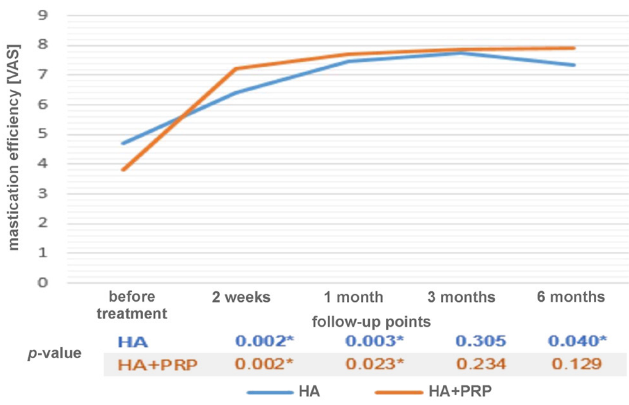

Fig. 5. Masticatory efficiency at the time of the study

* statistically significant.

Joint sounds

Similarly to what happened in the case of the pain variable, a decrease was observed in the first follow-up periods, the scores in the HA group increased significantly at 6 months, while in the HA+PRP group, they continued to decrease (Fig. 6).

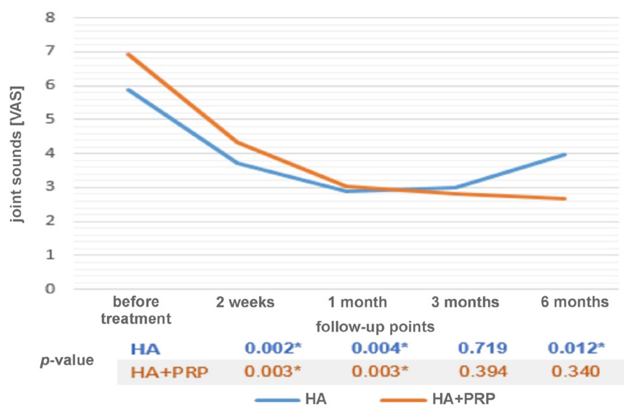

Fig. 6. Joint sounds at the time of the study

* statistically significant.

Maximum mouth opening

Measured in millimeters, there was an increase in the MMO values when comparing the periods before treatment–after 2 weeks with statistically significant differences. In the HA group, the mean MMO decreased when comparing the periods 3 months–1 month, also with a statistically significant difference. This did not happen in the HA+PRP group, where the mean MMO decreased slightly by about 0.16 at the end of the follow-up (Fig. 7).

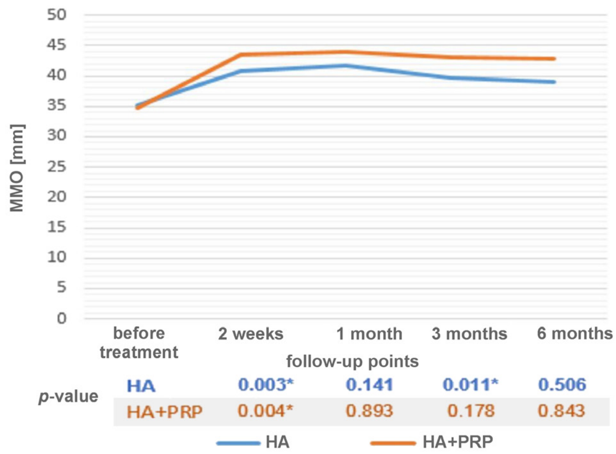

Fig. 7. Maximum mouth opening (MMO) at the time of the study

* statistically significant.

Functional limitation in the mandibular movement

As other study variables, functional limitation decreased with statistically significant differences in the first follow-up periods, but again increased at the end (at 6 months) in both groups. Still, the HA+PRP group recorded a slight increase, unlike the HA group, for which $p < 0.05$ (Fig. 8).

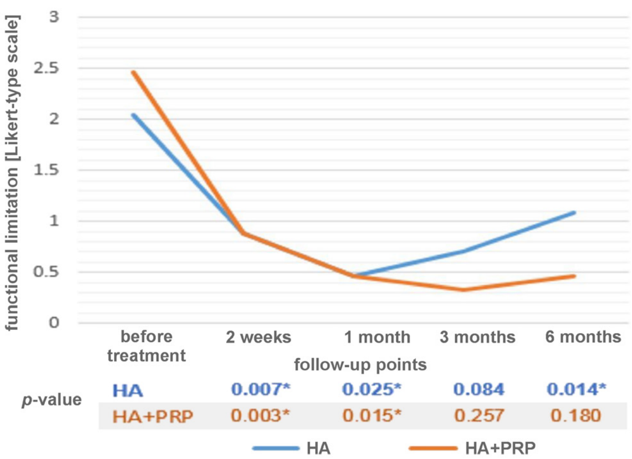

Fig. 8. Functional limitation of the mandibular movement at the time of the study

* statistically significant.

Intergroup comparison

Before treatment, it was noted that the means of some variables (pain, joint sounds and functional limitation) were higher in the HA+PRP group than in the HA group; they were lower at the end of the follow-up.

On the other hand, for other variables (mastication efficiency and MMO), the scores before treatment were lower in the HA+PRP group and became higher after 6 months.

The value of the significance level for all the variables of the 2 groups (HA and HA+PRP) in the pre-treatment period was greater than 0.5. There were statistically significant differences in all variables at the end of the follow-up except for the variable of joint sounds, which was similar in both groups (Table 2).

Table 2. Intergroup comparisons of the outcome variables (before treatment and after 6 months)

Variable	Before treatment		After 6 months	
	Z	*p*-value	*Z*	*p*-value
Pain at mastication	−0.465	0.671	−1.655	0.010*
Mastication efficiency	−1.072	0.291	−1.106	0.029*
Joint sounds	−1.399	0.178	−1.600	0.114
MMO	−0.144	0.887	−1.565	0.012*
Functional limitation	−1.194	0.266	−2.502	0.014*

* statistically significant.

Discussion

The evaluation of the treatment of TMDs focuses on 2 important areas: pain relief and restoring the mandibular function without limitation, which results in improvement in mastication efficiency.[3]

The intra-articular injection of HA provides many therapeutic benefits in the treatment of TMDs and serves as a minimally invasive procedure.[6] There is no need for surgical incisions and tissue dissection with this procedure, which decreases postoperative complications, such as facial nerve injuries, infection and severe pain.[1,14]

The use of PRP is also very beneficial, bringing significant and long-term improvement.[28]

The aim of the present study was to answer the question of whether the application of HA with PRP is superior to the HA injection alone in the treatment of TMDs. We started from the principle that HA has been commonly in use for the last 2 decades and has shown good efficacy; in addition to that, many authors have indicated improvement in the mandibular function without side effects associated to its use in treating TMDs.[1,7,8] Therefore, we designed our study so that the reference group were the patients who received HA alone.

The findings of our study suggest that HA was effective in alleviating the symptoms in all of the follow-up periods. However, in the long-term, at the 6-month follow-up point, HA+PRP provided better results.

The mean pain score decreased significantly in the HA group in all follow-up periods as compared to the pre-treatment period. It was 6.5 before treatment, 2.75 after 3 months and 3.5 at the end of follow-up, i.e., a significant decrease occurred. The HA+PRP group exhibited better performance as compared with the HA group, with the mean scores being 6.75, 3.25, 2.583, 2.375, and 2.250, consecutively in the follow-up periods.

Similarly to the previous variable, we found that the HA+PRP group had better mastication efficiency outcomes; the mean score at 6 months was 7.897 and 3.833 before treatment (p = 0.002), with a significant increase occurring in this group. In the HA group, an increase was achieved to a lesser extent and a decrease at the end of follow-up gave preference to the HA+PRP injection.

Although there were no statistically significant differences between the 2 groups concerning the joint sounds variable, the mean score in the HA group was 3 at 3 months and 3.958 at 6 months, i.e., an increase occurred, unlike in the HA+PRP group, where improvement continued until the end of follow-up.

The expected improvement was achieved in the variables MMO and functional limitation in both groups; however, preference was evident in the HA+PRP group.

At present, the published data about PRP and HA combined in the treatment of TMDs with which we could compare our findings and interpretations are limited.

We can discuss our study with regard to what researchers have stated about the use of HA alone or PRP alone. In their study, Gencer et al. reported that an injection of HA was superior to tenoxicam and betamethasone in relieving TMD complaints, including pain, which was reflected in an increase in mastication efficacy.[1] Kopp et al. reported similar findings associated with the clinical symptoms and dysfunction presented by patients.[15] They found that HA was safer and more suitable with regard to the risk of the progression of joint degeneration in comparison with corticosteroids.[15] On the other hand, PRP is an autologous solution; therefore, this method of treatment appears safe, especially that in recent years, this mixture (HA+PRP) has been studied in relation to the disorders of other body joints, such as the knee joint.[30]

Conclusions

The study findings suggest that the HA and PRP injection provides greater improvement in patients with TMDs as compared to the HA injection alone. This treatment method is safe enough as well as comfortable, and improves the quality of life of patients with TMDs.

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Rehabilitation in the treatment of mandibular condyle fractures

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Abstract

Background. The objective of rehabilitation after the treatment of a mandibular condyle fracture is to retrieve the effortless, symmetrical opening of the jaws with the preservation of appropriate movements to the sides, appropriate occlusion without a sense of tension in the mastication muscles or pain sensations.

Objectives. The aim of the article was to establish the conditions of the rehabilitation of temporomandibular joint (TMJ) after the surgical treatment of a fractured condyle.

Material and methods. The research featured 46 of patients treated surgically between January 2, 2017 and December 30, 2017. The open reduction and rigid internal fixation (ORIF) was the method mainly applied. Rehabilitation with the use of the Delphi technique was implemented in each patient. The assessment of the mastication organ was mostly performed at 3 and 6 weeks since the beginning of rehabilitation. In every patient, the interincisal distance was measured; in chosen cases, the range of condyle movement, the trajectory of condyle movement and the trajectory of the secant point during jaw opening were defined with the use of the Zebris® JMA device.

Results. In 45 patients, a full reposition and immobilization of 52 bone fragments of fractured mandibular condyles was obtained. The displacement of the midline of the mandible in occlusion or an incorrect occlusion contact determined the decision on the application of intermaxillary fixation in 8 patients for a period of 2 weeks. The minimal jaw opening of 40 mm was achieved in 41 patients after 6 weeks of rehabilitation. In 5 of the rest of the patients, rehabilitation had to be continued. The reassessment of the X-ray images and additional computed tomography (CT) diagnosis in three-dimensional (3D) reconstruction in those patients revealed the possible causes of difficulties in the restoration of the stomatognathic system functions – the injuries of the muscles or ligaments connected with the joint.

Conclusions. The extension of radiological diagnostics with a CT examination may improve the assessment of the restored TMJ function after the surgical treatment of a fractured mandibular condyle.

Key words: condylar fractures, self-control rehabilitation, temporomandibular joint injuries

Cite as
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Introduction

The frequency of fractures within the area of the mandibular condyle is assessed to be over 30% of all mandible fractures, and its incidence is in the range of 20–62%.[1–3] Mastering the surgical technique and enriching the range of tools in new solutions, including the endoscopic treatment of fractures, resulted in a more frequent use of open plate osteosynthesis in the fracture treatment as compared to the 20th century. The choice of a treatment method depends on the type of fracture, the displacement of the condyle head after the injury, the possibility of the reconstruction of the tooth contact in the opposite arches in habitual occlusion, and the decision of a patient who had been presented with the benefits and possible implications of closed reduction and maxillomandibular fixation (CRMMF) or open reduction and rigid internal fixation (ORIF).[4–7] The minimalization of damage resulting from ORIF, such as facial the nerve injury, visible scarring or salivary fistula formation, is possible with the use of the intraoral approach or endoscopic-assisted ORIF.[8,9] A full restoration of the stomatognathic system functions requires the implementation of rehabilitation after CRMMF as well as after ORIF.[10] Recommended rehabilitation techniques include, in addition to the auto-massage of the muscles involved in chewing, the introduction, at 2–3 days after surgery, of active jaw opening, the extension and mediotrusion of the jaw, performed by patients in front of the mirror to control the range and proper trajectory of movements. In the absence of functional progress at the 7th day after surgery, the authors recommend a passive, mild therapy involving stretching with fingers, spatulas or wooden wedges. The effect of rehabilitation depends to a large extent on patient self-discipline.[5,11,12]

The beneficial impact of functional rehabilitation of the oral cavity and speech rehabilitation on the reconstruction of bone tissue after a mandibular condyle fracture is commonly emphasised.[5,12] Due to a mechanical load within the area of bone fracture, a lesser loss of bone substance in the bone fragment circuit is observed as well as an increase in the bone number between the bone fragments within the physiological limits. In these conditions, the osteoclast resorption in the external sites of anatomical growth modeling is inhibited and new, immature scaffolding is created, including de novo a rich supply of capillaries.[13]

A lack of standardized programs of rehabilitation of the oral cavity functions after a mandibular condyle fracture results in difficulties in the assessment of treatment outcomes. In 2016, van der Merve and Barnes presented the Delphi technique, which is a complex method of temporomandibular joint (TMJ) rehabilitation after a mandibular condyle fracture.[14] The Delphi technique emerged as an effect of the compatible assessment of the intervention programs regarding the physical therapy of patients with mandibular condyle fractures, performed by international experts experienced in the field of maxillofacial surgery, dental surgery and physical therapy.[15]

In the patients treated due to a mandibular condyle fracture in 2017, we applied post-surgical rehabilitation in accordance with the Delphi technique. The methods applied by us earlier, which focused on the directed activization of the stomatognathic system muscles, were also similar to the ones that are included in the Delphi technique. A precisely defined time of use, the number of repetitions of particular exercises and the assessment of rehabilitation effects were the features acquired by us from the specialists applying the Delphi technique in 2016. We did not encounter any scientific publication on the results of the rehabilitation of the oral cavity functions with the use of the Delphi technique. The difficulties recognized in rehabilitation are presented in this article.

Objectives

The main objective of the present study was to implement the Delphi technique in the mastication organ rehabilitation in patients after the surgical treatment of mandibular condyle fractures, and recording the conditions and results of the rehabilitation.

Material and methods

The research was approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin, Poland (approval No. KB-0012/30/13). All of the patients included in the study gave written consent for their diagnostic examination results, the obtained surgical treatment and rehabilitation goals to be published.

The research featured 46 of patients aged 17–49 years (the mean age: 29.2 years) treated surgically between January 2, 2017 and December 30, 2017 due to a mandibular condyle fracture. Diagnosis was made on the basis of a clinical examination, a pantomographic X-ray image, and a computed tomography (CT) scan of the facial part of the skull. Open reduction and rigid internal fixation was applied, and in some cases, closed reduction and maxillomandibular fixation (CRMMF) was used within a period of 2 weeks. Table 1 presents all the information on the treatment and rehabilitation of the patients treated due to a mandibular condyle fracture.

Rehabilitation with the use of Delphi technique was implemented in every patient who had given conscious consent for the complementation of the applied surgical treatment with the directed activization of the stomatognathic system muscles. Rehabilitation was commenced at the 2nd day post-surgically during the patient's stay at the ward under the surgeon's care. Patients in whom there was a necessity of the MMF application, after the

Table 1. Fractures of the condylar process treated between January 2, 2017 and December 30, 2017

Condylar process fracture	Number of fractures *n*	Treatment		Duration of rehabilitation [weeks] (Delphi technique 2016)
		ORIF	CRMMF	
Isolated fracture unilateral bilateral	 14 6	 13 4	 1 2	 3–6 6
Coexisting with mandibular fractures on the same side	2	2	–	3–6
Coexisting with mandibular fractures on the opposite side	19	18	2	6
Coexisting with bilateral mandibular fractures	8	8	2	>6 and <52
Coexisting with bone fractures of the facial part of the cranium	3	2 defragmented head of the mandible removed – 1 patient	1	>6 and <52
Total	52	47	8	3–52

ORIF – open reduction and rigid internal fixation; CRMMF – closed reduction and maxillomandibular fixation.

fixation removal in the ambulatory conditions, were given detailed information on the self-performance of the exercises designed to restore the TMJ functions. Suggestions concerning food consistency and the size of a mouthful were given to the patients on discharge from hospital or after intermaxillary fixation. Table 2 presents exercises with the suggestions concerning their duration and the number of repetitions proposed within the Delphi technique.

The assessment of the mastication organ functions was performed after 3, 6 and 52 weeks since the beginning of rehabilitation. The interincisal distance was measured in each patient; in chosen cases, the range of condyle movement, the trajectory of condyles movement and the trajectory of the secant point during jaw opening were defined with the use of the Zebris® JMA device.

Results

Among 148 of patients treated surgically due to a mandible fracture in 2017, 46 patients (31%) were treated due to 52 mandibular condyle fractures. In the case of 47 of the fractures of the mandibular condyles, ORIF was applied. In all of the 47 cases, the reposition and stabilization of bone fragments was achieved. Maxillomandibular fixation was applied in 8 of the patients as complementary treatment for ORIF in bilateral fractures, where dental occlusion was different from the assumed.

After 6 weeks of rehabilitation, 41 of the 46 patients (89.1%) assessed the range of their movements within the area of TMJ as satisfying; the interincisal distance was at least 34 mm. Regardless of whether the patients were used to self-control (active jaw opening performed by patients in front of the mirror to control the range and proper trajectory of movements) for a long time or not, they still assessed the width of jaw opening and its symmetry. Such self-control was also an exercise. At 52 weeks, the patients achieved an opening of at least 42 mm.

The disturbed functions of TMJ recorded after 6 weeks of rehabilitation – lateral deviation in mouth opening, malocclusion or mouth opening <34 mm – occurred in less than ¼ of the patients. Table 3 presents the information on the disturbances of the TMJ functions after surgical treatment and after 6 weeks of rehabilitation due to a mandibular condyle fracture.

Mandibular deviation during opening was present in 9 of the patients and it was in the range of 8–11 mm. Jaw opening in 4 out of 9 of the patients was sufficient. In all of the 9 patients, exercises for jaw opening in front of the mirror, including gentle hand pressure, which works against mandibular deviation from the midline during jaw opening, were suggested.

Occlusion disturbances were recorded in 8 of the patients. Mandibular deviation from the midline in these patients was 1–2 mm. Jaw opening in 3 out of 8 of the patients was sufficient. The revision of the occlusion performed in the 3 patients improved the conditions in the

Table 2. Exercises and dosages according to the Delphi questionnaire

Exercise	Repetitions at one time	Repetitions per day
Isometric jaw contractions (held for 5 s)	5 contractions per muscle	3
Jaw opening	5 movements	3
Lateral jaw movement	5 movements	3
Jaw protrusion	5 movements	3
Stretch (held for 5 s)	5 movements	3

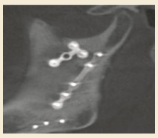
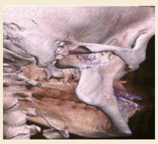
Table 3. Disturbed functions of the temporomandibular joint (TMJ) in 46 patients after a condylar process fracture, open reduction and rigid internal fixation (ORIF) and 6 weeks of rehabilitation

Disturbances in TMJ functions	Number of patients *n*
Lateral deviation in mouth opening	9
Malocclusion	8
Mouth opening <34 mm	5
Pain in TMJ	5

stomatognathic system. The results were assessed by the patients as positive. In the remaining 5 patients, muscle exercises in front of the mirror were continued. After 52 weeks of rehabilitation, mandibular deviation was present in 2 of the patients and it amounted to 1 mm. The patients accepted the situation and they did not report any problems at rest nor during mastication.

In 5 of the patients, the interincisal distance was of 27–30 mm. These patients complained about painful jaw opening limitation. Spontaneously implemented by these patients analgetic treatment was applied once a day in the morning, when the pain was the greatest. In these patients, the X-ray imaging results were revised and new CT scans were performed in three-dimensional (3D) reconstruction (Table 4). The rehabilitation technique applied earlier was maintained. After 52 weeks of rehabilitation, a jaw opening of 34–44 mm was recorded in these patients (Fig. 1).

Table 4. X-ray images of the fractured condylar processes in the patients in whom the necessary rehabilitation lasted more than 6 weeks

Patient	Chosen X-ray image of the condylar process	Fracture description	Damage to the ligaments or muscles
QX1		multi-fragmented fracture of the mandible, fracture of both condylar processes, fracture of both pterygoid processes, rotation of the left pterygoid process	avulsion of the temporomandibular ligament, damaged attachment of the left temporal muscle
QX2			
ZY		dislocation in TMJ, multi-fragmented fracture of the condylar process, displacement of the fragments mid-linear and forward	avulsion of the temporomandibular ligament and the retrodiscal ligament
XY		multi-fragmented fracture of the mandible, recurring dislocations in TMJ	avulsion of the upper part of the lateral pterygoid muscle with a fragment of the condylar head and the lateral plate of the pterygoid process of the sphenoid bone, avulsion of the lateral ligament
VX		vertical fracture of the mandible branch, dislocation in TMJ	avulsion of the sphenoid-mandibular ligament
QV		multi-fragmented fracture of the mandible, fracture of the condylar process, dislocation in TMJ	avulsion of the temporomandibular ligament

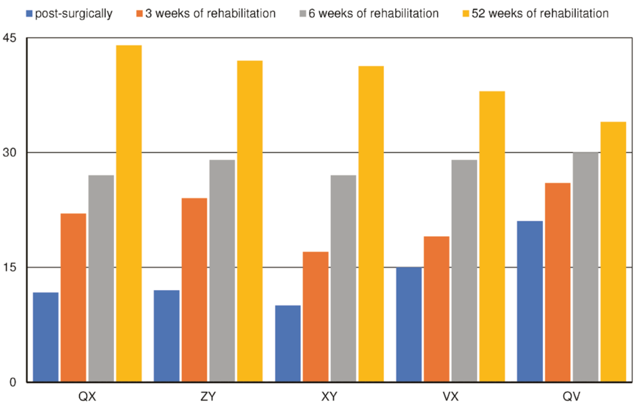


Fig. 1. Interincisal distance in 5 of the patients in whom rehabilitation was prolonged

In patient XY, jaw opening improvement was taking place at the slowest rate. After 6 weeks of rehabilitation, 13 weeks post injury, jaw opening in the patient expanded from 10 to 27 mm. The late beginning of rehabilitation was associated with dislocation in TMJ, which primarily occurred due to a complex injury – a multi-fragmented fracture within the mental region, a bilateral fracture within the area of the mandible angles, a right-side fracture of the condyle process and the styloid process, with the knock-out of lower central incisors (Fig. 2). The displacement of TMJ occurred for the 2^{nd} and 3^{rd} time in the patient, in whom the fragments of the mandible were stabilized through the use of ORIF, and finally permanent MMF was applied after placing a partial mobile prosthesis in the oral cavity, which recreated lower central incisors. Anxiety about the renewal of the TMJ displacement probably retarded full commitment to rehabilitation.

Changes in the range and shape of the trajectory of the condyle process during opening/closing the mouth recorded in patient XY during the 2^{nd} half-year of rehabilitation are shown in Fig. 3 and Fig. 4. The record made after 26 weeks of rehabilitation showed the shortening

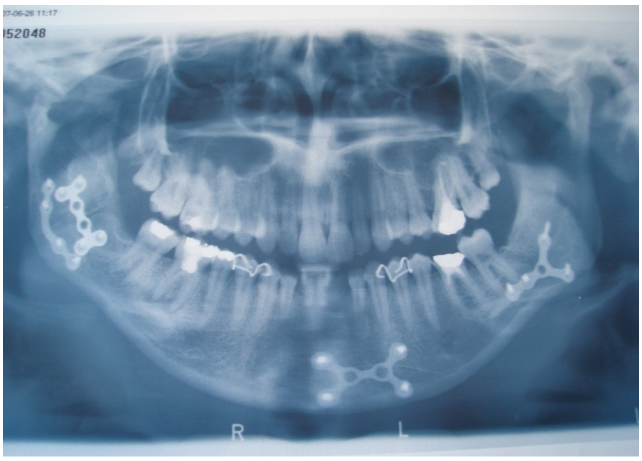


Fig. 2. Pantomogram of female patient XY – a multi-fragmented fracture of the mandible within the mental region, within both angles, a fracture of the right condylar process as well as the knock-out of lower central incisors

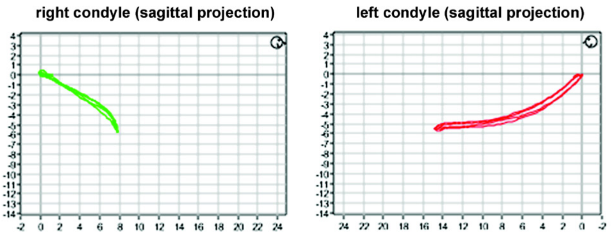


Fig. 3. Range and shape of the condylar track during the opening/closing of the right TMJ and the left TMJ after 26 weeks of rehabilitation

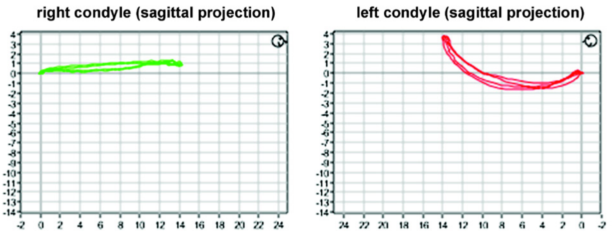


Fig. 4. Range and shape of the condylar track during the opening/closing of the right TMJ and the left TMJ after 52 weeks of rehabilitation

and flattening of the trajectory of the fractured and displaced right condyle process as compared to the left side. Another record, after 52 weeks of rehabilitation, showed a longer and flatter trajectory of the right condyle process than in the previous examination.

A change in the trajectory of the incisal point during mouth opening, which occurred during the 2nd half-year of rehabilitation is shown in Fig. 5. The record made after 26 weeks of rehabilitation showed a mandible deviation of 5 mm toward the fractured and displaced condyle, throughout the whole movement of opening, especially during its 2nd phase. Another record, made after 52 weeks of rehabilitation, showed a symmetrical trajectory of conducting the mandible with a 3-millimeter deviation toward the contralateral side to the fractured and displaced condyle, at the end of the 2nd phase of the opening movement.

The CT scan of the skull in 3D reconstruction performed after 52 weeks of rehabilitation showed the deformation of the right condylar process and the cup on the side (Fig. 6).

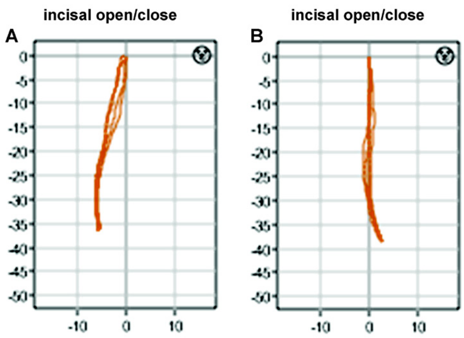


Fig. 5. Trajectory of the incisal point during jaw opening/closing in female patient XY

A – after 26 weeks of rehabilitation; B – after 52 weeks of rehabilitation.

Changes in the shape of the condyle head arose during a year's rehabilitation of the oral cavity functions. At that time, the head of the condyle grew together with the torn off fragment of the head of condyle; the broken off side plate of the pterygoid process was still visible as a free fragment.

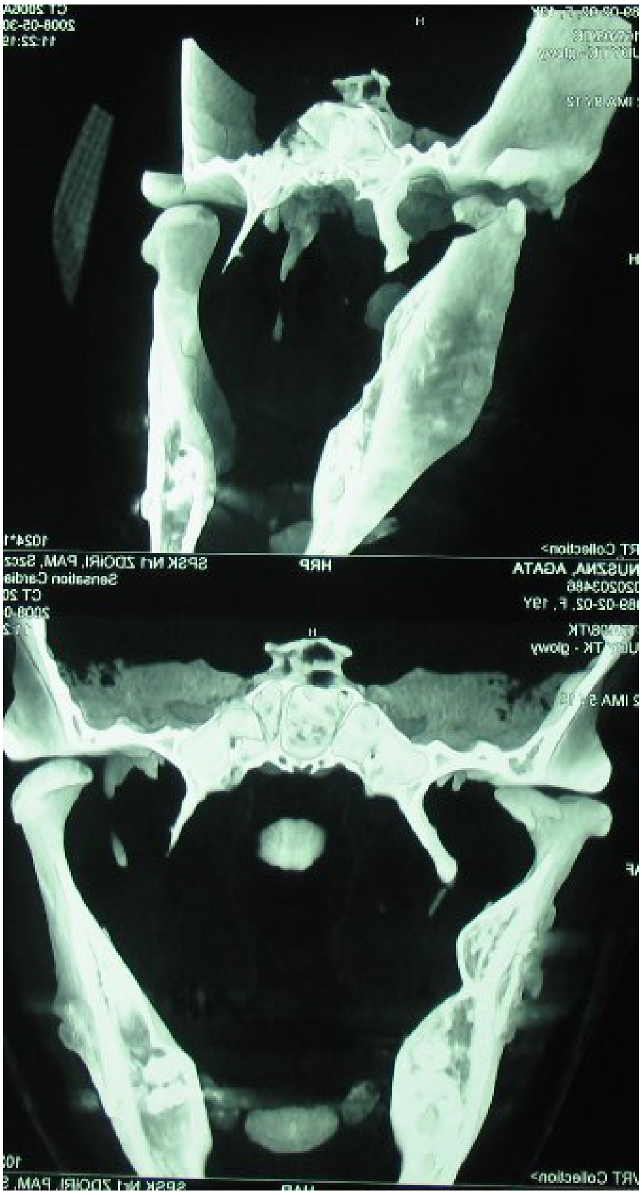


Fig. 6. Computed tomography (CT) scan in three-dimensional (3D) reconstruction in female patient XY after 52 weeks of rehabilitation, 59 weeks after the injury

Discussion

The Delphi technique was approved as a procedure of rehabilitation in our facility on the basis of the fact that the directed activization of the stomatognathic system muscles – similar to the Delphi technique – had been used for the purpose of rehabilitation after a condyle process fracture for many years. Independently of the treatment method applied (ORIF or CRMMF), post-surgical autocorrection and self-control was suggested to each patient. A check-up examination (no complications) was performed a week after ORIF or after the removal of intermaxillary fixation. Another examination, after 3 weeks of rehabilitation, was the 1st assessment of the restoration of the oral cavity functions; it was also the moment when the decision on further rehabilitation was made.

According to the data gathered by van der Merwe and Barnes, the disturbances of the stomatognathic system functions after ORIF performed due to condyle process fractures affect 0–49% of patients, and after the closed treatment – 11–49% of patients.[14] It may be assumed that more than 50% of patients after the treatment of a mandibular condyle process fracture need the rehabilitation of the oral cavity functions. Self-management is the basic intervention which ensures simple reversible therapy. However, there is no unambiguous, simple definition of self-management in the literature. There is a dominant opinion that all of the self-management programs have similar general goals, and that the goals may be supplemented with further goals and supporting modules, specifically for the needs reported by patients, i.e., focused mainly on the improvement of mouth opening.[15]

Mouth opening lesser than expected by the patient is the main problem after a condylar process fracture. However, there are some jaw opening widths referred to as a norm; according to Agraval et al., it is 50 mm,[16] and according to Cox and Walker, the minimum is 34 mm.[17] A patient's sensation that they can effortlessly or painlessly, and accordingly to their assumptions, open and close their mouth is the most important. The cause of mouth opening limitation may be directly associated with the injury effect. The limitation may also results in the necessity of applying intermaxillary fixation for some time. Determining the cause seems to be important for effective rehabilitation. Pre-surgical orthopantomograms and sagittal CT scans in the described group of patients seem to be insufficient for the assessment of the post-injury changes concerning bone and soft elements of TMJ. Condylar process fractures are perceived by the authors specialized in the field as difficult for imagining due to the overlapping of many anatomical structures on the condylar process. An oral pantomogram, posteroanterior X-ray images of the mandible, and alternatively a lateral oblique view, are reliable imagining examinations implemented in the diagnosis of fractured craniofacial bones; CT, and especially CT in 3D reconstruction, are the methods of choice in the diagnosis of condylar process fractures.[3,18] According to Mueller et al., spiral CT gives a 100% chance of defining a mandible fracture, while panoramic tomography gives an 86% chance.[19]

The symmetry of jaw opening and closing, or more precisely – a lack of the symmetry, is another problem for the patients rehabilitated after a mandibular condyle process fracture. Performing the suggested exercises by patients in front of a mirror results in the multiple repetitions of the desirable symmetrical jaw opening and closing. The majority of the 46 patients treated by us (over 80%) presented with the stabilized central closure and symmetrical opening of the jaws after 6 weeks of rehabilitation; in 3 of the patients, a closure correction was performed due to a mandible deviation from the midline of 1–2 mm in closure. These patients performed the movement of opening symmetrically. In 19% of the patients presented in Table 3, there was a significant asymmetry (3–11 mm) during the movement of jaw opening and a slight asymmetry (1–2 mm) in closure. These patients were encouraged to further rehabilitation.

A similar frequency of mandible deviation during mouth opening and a 3 times lower frequency of inappropriate closure at 6 weeks after ORIF due to a mandibular condyle process fracture are reported by Cuéllar et al.[20] The authors, based on 6 randomized trials involving 288 patients, suggest a lower probability of malocclusion, lateral deviation in mouth opening and pain in TMJ after surgical management than after conservative management. According to Villarreal et al., the improvement of the stomatognathic system functions achieved by means of ORIF was greater than in the case of CRMMF; however, open treatment increased the frequency of the post-surgical malformations of the condyles and mandible asymmetry.[21] There is a need for standardizing the methods of assessment of the functions of the stomatognathic system in order to enable a reliable comparison of treatment results.[22]

However, the self-assessment of the TMJ functions performed by the patient is the basis of TMJ rehabilitation accordingly to the Delphi technique. The use of electronic recording systems supported by computers (Zebris® JMA; Drive Medical, Isny im Allgäu, Germany) and modern radiology techniques allows for the assessment of the efficiency of the therapy, especially when rehabilitation does not provide desirable effects within the provided time. According to Kijak et al., recording changes in the TMJ functions over time is beneficial for understanding the occurring improvement and motivating for further exercises.[23]

Tooth loss as a result of an injury, especially the loss of lower incisors after a mandible fracture within the mental region, may cause the loss of space in the dental arch. In a study dealing with the issue, a greater frequency of space loss was shown in patients who – apart from multiple mandible fractures – also had fractures of the condylar processes.[24] According to the authors, maintaining the space prevents from the complications associated with its reduction. Difficulties in prosthetic treatment after CRMMF due to mandibular condyles fractures are associated with restricted jaw opening and an increased tension of the muscles of the stomatognathic system, and the prognosis for a full recovery of its functions is uncertain.[25] It is then favorable to perform a prosthesis as soon as possible to decrease the possibility of the expected difficulties. It is especially important for maintaining a correct occlusive support. It was demonstrated on the basis of the axiographic examinations of patients in whom the medical problem was partial anodontia, the pathological attrition of other teeth and the TMJ dysfunctions.[26]

In our female patient XY, lower central incisors knocked out during a car accident could not be replanted because they were lost in the damaged car. A partial movable prosthesis made 5 weeks after the injury maintained the space in the dental arch in place of the knocked out incisors and participated in dental occlusion until permanent intermaxillary fixation was put.

The late exposure (CT with 3D reconstruction was performed 5 weeks after the injury) of the avulsion of the lateral pterygoid muscle with a fragment of the head of the condylar process and the lateral plate of the pterygoid process was not only a diagnostic issue for us. Questions regarding further treatment have only partially been answered in experimental research.[27,28] Both of the above-mentioned studies showed the lateral pterygoid muscle as an element which stimulates distractive osteogenesis after a condylar process fracture, and which along with the injured joint disc may be an important factor in the etiology of TMJ ankylosis.[27,28] In case of disruption of the muscle, the stimulation of osteogenesis will not take place.

In the case of our female patient XY, the late diagnosis of the avulsion of the lateral pterygoid muscle was the cause of the recurring dislocation of the condylar process. It was probable that the muscle avulsion and functional rehabilitation prevented TMJ from getting stiff.

Conclusions

Autocorrection and self-control were the right methods to improve the TMJ functions after the surgical treatment of condylar process fractures. The external control exercised by a maxillofacial surgeon enabled the assessment of rehabilitation efficiency as well as signalized the need for finding circumstances that inhibit the restoration of the TMJ functions. Further assessment of the radiological documentation, supplemented with CT with 3D reconstruction validated previous assumptions that the condylar process fracture was accompanied by serious injuries of muscles and joint ligaments. Our observations point to the fact that rehabilitation after surgical treatment of a condylar process fracture may be long-lasting and it should be started as soon as possible.

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Original papers

Effects of non-surgical periodontal treatment in rheumatoid arthritis patients: A randomized clinical trial

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Abstract

Background. The periodontal condition has a reciprocal relationship with rheumatoid arthritis (RA). Rheumatoid arthritis patients are reported to present with more serious periodontal disease (PD) as compared to non-RA patients.

Objectives. This study aimed to evaluate the effects of non-surgical periodontal treatment on Vietnamese patients with active RA and PD, where the clinical characteristics and serum indices of the patients were of interest.

Material and methods. We conducted a randomized clinical trial (RCT) on 82 RA patients with PD. The patients were randomly divided into 2 groups: the intervention group, consisting of patients who received oral hygiene instructions, scaling and root planing; and the control group, consisting of patients who received oral hygiene instructions only. Both groups received the same treatment plan for RA. The Disease Activity Score 28 based on C-reactive protein (DAS28-CRP), disease activity classification, rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), anti-citrullinated protein autoantibodies (ACPAs), and C-reactive protein (CRP) were monitored, with the measurements taken at 3 months and 6 months following the treatment.

Results. The 2 groups exhibited similar parameters at baseline. In the intervention group, DAS28-CRP and disease activity classification were significantly reduced at 3 months after treatment as compared to the baseline data. At 6 months following the treatment there was a significant decrease in ESR, ACPAs and DAS28-CRP in the intervention group, while the control group showed a decrease only in ACPAs. Further, when comparing the intervention and control groups at 6 months following the treatment, there were no differences between the groups in the ACPAs, RF and CRP serum levels.

Conclusions. Non-surgical periodontal treatment can significantly reduce DAS28-CRP, disease activity classification, ESR, and the ACPAs level in serum, and can be applied to reduce RA severity in RA patients with PD.

Key words: rheumatoid arthritis, inflammation, periodontitis

Introduction

Rheumatoid arthritis (RA) patients usually have periodontitis and, in comparison with those without RA, also have a worse periodontal condition.[1,2] Periodontitis is a common chronic infection, with a global incidence in adults varying from 10% to 60%, depending on different diagnostic criteria.[3] Reports have indicated a high incidence of diabetes mellitus, atherosclerosis, myocardial infarction, stroke, and RA in patients with periodontal disease (PD).[4–7] In our recent report on the association between PD and RA in Vietnamese patients, we found that the periodontal condition of RA patients is more serious as compared to non-RA patients.[8] There was an association between the periodontal condition and clinical symptoms, such as the biochemical and immunological characteristics of RA.[8]

Rheumatoid arthritis is a common autoimmune disease; its chronic progression includes symptoms at joints and other body parts with varying severity, which can lead to the weakening of bones and connective tissues.[9] Studies have shown that patients with RA are at high risk of cardiovascular diseases and malignancy.[10,11] Rheumatoid arthritis is prevalent worldwide, with an incidence rate of 0.5–1% in adults. In Vietnam, 0.5% of the population has RA. Among hospitalized patients, the RA rate is as high as 20%. In particular, 70–80% of patients are female, and 60–70% of them are over 30 years old. The disease is hereditary in some cases.[12] Previous clinical studies show different results with regard to the role of non-surgical periodontal treatment in RA. In their research, de Noronha Pinho et al. found that the relationship between the RA and PD disease activities was not clear.[13] However, it is evident that due to periodontal treatment, it is possible to control inflammation and avoid tooth extraction. A recent study by Zhao et al. demonstrated that RA patients with PD who received non-surgical treatment showed improvement in the clinical outcome for RA.[14] Recently, a new classification for PD has been developed based on previous classifications.[15,16] Machtei et al.[17] defined the term 'established periodontitis' with regard to 'periodontitis', which was used in clinical studies,[18,19] and 'severe periodontitis', referred to by the Centers for Disease Control and Prevention (CDC) in partnership with the American Academy of Periodontology (AAP).[20]

In this study, we evaluated the effects of non-surgical periodontal treatment on the clinical characteristics and serum indices in 82 Vietnamese patients with active RA and PD, using Machtei's classification. The Disease Activity Score 28 based on C-reactive protein (DAS28-CRP), disease activity classification, rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), anti-citrullinated protein autoantibodies (ACPAs), and C-reactive protein (CRP) were measured and monitored at baseline, and at 3 and 6 months following the periodontal treatment.

Material and methods

Study design

We conducted a clinical intervention study on RA patients with periodontitis who were treated at the Department of Rheumatology, Cho Ray Hospital, Ho Chi Minh City, Vietnam, from August 2013 to March 2015. The trial registration number is ISRCTN40789708 at www.isrctn.org.

Eligibility criteria

Rheumatoid arthritis patients with PD were recruited in the present trial in the same way as in our previous study.[8] Rheumatoid arthritis was diagnosed using the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2010 guidelines,[21] while the periodontal condition was determined according to the criteria specified by Machtei et al.,[17] with more than 4 real teeth (regardless of the largest third molars). All patients had established RA (>12 months), and were treated with disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. The exclusion criteria were as follows: patients under the age of 30 years; RA patients with other conditions, such as polymyalgia, gout, pseudogout, spinal stiffness, Sjögren's syndrome, diabetes mellitus, and malignant diseases; patients who had recently received periodontal treatment (3 months or less prior to our study); and patients who were pregnant or breastfeeding.

Randomization

Independent staff members randomly assigned patients into the intervention or control group, using sealed envelopes. Each envelope was marked with a '0' or a '1'. A patient was assigned to the control group if their envelope had a '0'; otherwise, the patient was assigned to the experimental group. A separate envelope was used for each kind of treatment, and the type of treatment for each patient was documented.

Blinding

Whether a patient belonged to the intervention or control group was fully disclosed to the patient and the clinician treating PD (the main investigator). Due to the nature of the intervention, blinding was not applied to the patients and the main investigator. The outcome was blinded to the assessing investigators and the statistician.

Interventions

Both the control and intervention group were treated with conventional regimens. The control group received only oral hygiene instructions, while the treatment group received an advanced periodontal treatment intervention consisting of supragingival scaling and root planing, the effectiveness of which was evaluated after 3 and 6 months, which was followed by retreating. The research team consisted of a periodontist, who recorded the periodontal indices, a rheumatologist, who assessed the swollen joints, and an assistant, who recorded the information. The researchers who treated the RA patients for periodontitis were not involved in the periodontal, joint or other clinical examinations. The team members were trained by experts before the study, achieving consistency from 80% to 90.4%.

Outcomes

Periodontal disease assessment

The assessment of PD was conducted in a similar manner as described in of our previous study.[8] The periodontal indices included the plaque index (PlI), the gingival index (GI), the percentage of sites with bleeding on probing (%BOP), probing pocket depth (PPD), and clinical attachment loss (CAL), evaluated at 6 positions in all teeth.[22,23]

Rheumatoid arthritis clinical assessment

Rheumatoid arthritis disease activity was measured as in our previous study.[8] We assessed RA disease activity based on CRP (DAS28-CRP), with the following equation (Equation 1):

$$\text{DAS28-CRP} = 0.56 \times \sqrt{\text{TJC28}} + 0.28 \times \sqrt{\text{SJC28}} + 0.36 \times \ln(\text{CRP} + 1) + 0.014 \times \text{GH} + 0.96 \quad (1)$$

where:
TJC28 – tender joint count (0–28);
SJC28 – swollen joint count (0–28);
CRP – C-reactive protein level [mg/L];
GH – patient's global health self-assessment by means of the visual analog scale (VAS).[24]

The determination of the DAS28-CRP score regards the pain and swelling of 28 joints, comprising shoulder joints, elbow joints, wrist joints, and knee joints. We applied the tool available at http://www.4s-dawn.com/DAS28 to compute the value of DAS28-CRP. Then, DAS28-CRP was classified as remission (DAS28-CRP < 2.3), low (2.3 ≤ DAS28-CRP < 2.7), moderate (2.7 ≤ DAS28-CRP < 4.1), or high disease activity (DAS28-CRP ≥ 4.1).

Measurement of ESR, and the RF, ACPAs and CRP levels in serum

We determined the serum concentration of RF and CRP with the use of the latex particle-enhanced method.[8] An RF serum value of <12 IU/mL was defined as normal (negative) and >12 IU/mL as positive. The CRP serum level was defined as normal if it was <6 mg/L. The serum level of ACPAs was determined by means of the sensitive enzyme-linked immunosorbent assay (ELISA). The ACPAs serum level was defined as normal or negative (<25 IU/mL) or positive (≥25 IU/mL). Erythrocyte sedimentation rate was measured using the flow through a capillary tube. The normal serum ESR was recorded at 5–10 mm/h.

Sample size

Eighty-two patients with RA were randomly split into 2 groups: treatment group ($n = 41$); and control group ($n = 41$). The sample size was calculated as follows (Equation 2):

$$n = \frac{\sigma^2 \left(Z_{(1-\alpha/2)} + Z_{(1-\beta)}\right)^2}{\left(\mu_1 - \mu_0\right)^2} \quad (2)$$

where:
n – sample size;
σ^2 – population variance;
$Z_{(1-\alpha/2)}$ – critical value of normal distribution at $\alpha/2$;
$Z_{(1-\beta)}$ – critical value of normal distribution at β;
μ_1 and μ_0 – means of the 2 groups.

We first derived the value of $(\mu_1 - \mu_0)$ from a study by Ortiz et al.[25] According to them, the average Disease Activity Score 28 (DAS28) in RA patients after periodontal treatment was 3.51 ±1.11, with a change in DAS28 of 0.6, defined as treatment response.[21,25] Thus, we used a value of 0.6 for $(\mu_1 - \mu_0)$. Using $\alpha = 5\%$ and a power of 80% in Equation 2, we obtained a sample size of 27 ($n = [1.11(1.96 + 0.84)^2] / (0.6 - 0)^2 = 27$) patients for each group. According to the internal statistics provided by Cho Ray Hospital, 30–35% of patients cancel the re-examination. Therefore, in order to account for a sampling error and the potential sample size reduction, a sample size of 41 patients was used for our study.

Research ethics

The study was conducted in full accordance with the World Medical Association Declaration of Helsinki from 2008, and was approved by the Ethics Committee of the University of Medicine and Pharmacy at Ho Chi Minh City in Vietnam (No. 1781/DHYD-HD). Written informed consent was obtained from all participants prior to inclusion. At the end of the study, patients from the control group also received the same periodontal treatment as the treatment group.

Statistical analyses

We reported the mean (*M*) and standard deviation (*SD*) values for normally distributed data with the Shapiro–Wilk test, and the median (*Me*) and interquartile range (*IQR*) values otherwise. Differences between the 2 groups were assessed using the independent samples *t* test. The Mann–Whitney *U* test was applied to *Me* and *IQR*. For intergroup comparison, the quantitative parameter values between the groups were assessed with the Wilcoxon test. The χ^2 test and Fisher's test were used for rate comparison. The odds ratios (*OR*s) and 95% confidence intervals (*CI*s) were also reported. Statistical significance was defined at $p < 0.05$. We performed statistical analyses using the Stata statistical software, v. 13.0 (StataCorp, College Station, USA).

Results

Parameters of the 2 groups at baseline

The flow diagram of the study is shown in Fig. 1. Between the control and treatment groups there were no differences in the distributions of age, gender, place of residence, occupation, smoking status, number of teeth examined, and duration of RA. Among the treatment group, the average age was 52.9 ±8.2 years and the proportion of women was 88%. Among the control group, the average age was 51.9 ±9.0 years and the proportion of women was 93% (Table 1). Besides, DAS28-CRP, ESR, and the serum levels of RF, CRP and ACPAs at baseline were also similar in the 2 groups. Around 2–3 patients per group were lost at 6 months due to not coming back (missing data).

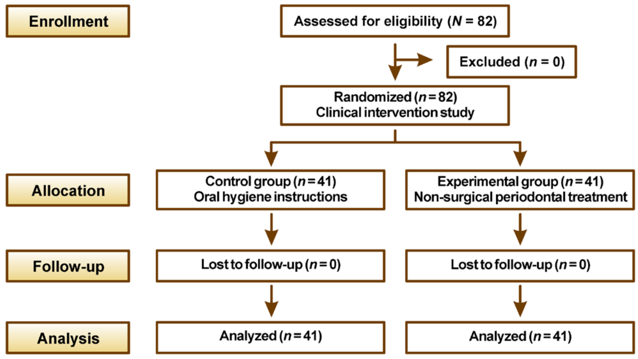

Fig. 1. Flow diagram of the study

Around 2–3 patients per group were lost at 6 months due to not coming back (missing data).

Table 1. Characteristics of the study groups

Parameter	Treatment group n = 41	Control group n = 41	*p*-value	*OR* (95% *CI*)
Gender female male *n* (%)	 36 (87.8) 5 (12.2)	 38 (92.7) 3 (7.3)	0.712[a]	 0.57 (0.13–2.55) 1
Age [years] *M* ±*SD*	52.9 ±8.2	51.9 ±9.0	0.590[b]	1.01 (0.96–1.07)
Age group middle age (31–60 years) old age (>60 years) *n* (%)	 34 (82.9) 7 (17.1)	 34 (82.9) 7 (17.1)	0.999[b]	 1 1
Residence Ho Chi Minh City other *n* (%)	 1 (2.4) 40 (97.6)	 5 (12.2) 36 (87.8)	0.201[a]	 0.18 (0.02–1.61) 1
Smoking yes no *n* (%)	 3 (7.3) 38 (92.7)	 3 (7.3) 38 (92.7)	0.999[a]	 1.00 (0.19–5.27) 1
Occupation farmer worker non-manual worker housewife *n* (%)	 14 (34.1) 4 (9.8) 11 (26.8) 12 (29.3)	 15 (36.6) 8 (19.5) 12 (29.3) 6 (14.6)	0.351[a]	 1 0.54 (0.13–2.18) 0.98 (0.33–2.94) 2.14 (0.63–7.27)
Number of remaining teeth *Me (IQR)*	22 (20–24)	22 (13–25)	0.474[c]	1.07 (0.98–1.16)
Duration of RA [years] *Me (IQR)*	3.5 (2–9)	3 (2–7)	0.950[c]	0.98 (0.91–1.05)

OR – odds ratio; *CI* – confidence interval; *M* – mean; *SD* – standard deviation; *Me* – median; *IQR* – interquartile range; RA – rheumatoid arthritis; [a] Fisher's test; [b] *t* test; [c] Wilcoxon test. 95% *CI* was not applied in case of *OR* = 1.

Changes between the groups in the clinical parameters of periodontitis after 3 and 6 months in relation to baseline

On the 1st day of the examination, both the control and treatment groups were similar in terms of PlI, %BOP, periodontitis severity, and the CAL level (Table 2). On the other hand, GI, PPD and CAL were higher in the treatment group than in the control group ($p < 0.05$). Patients with RA in the treatment group tended to have more severe soft tissue inflammation than patients in the control group. After 3 months of treatment for periodontitis, most of the indices, including GI, %BOP, PPD, and CAL, significantly improved in the treatment group ($p < 0.001$). These indices in the treatment group continued to decline after 6 months ($p < 0.001$) as compared to baseline. The difference between the 2 groups became more evident as measured at 3 and then at 6 months ($p < 0.001$). After 6 months, the *OR*s for PlI, GI, %BOP, and PPD between the treatment and control groups were 0.80, 0.06, 0.32, and 0.02, respectively. The intensive periodontal treatment improved the periodontal status in patients with RA. Meanwhile, these indices in the control group stayed almost unchanged as compared to baseline.

Comparison of the changes in the clinical and paraclinical indices of rheumatoid arthritis after 3 and 6 months between the groups

After 3 months of periodontitis treatment, the clinical indicator of RA (DAS28-CRP) decreased in the control group; however, the difference was not statistically significant ($p > 0.05$). After 6 months, all indicators decreased in the treatment group as compared to the control group, and the difference was statistically significant (Table 3). The treatment group showed reduced DAS28-CRP ($p < 0.001$) and disease activity level ($p < 0.001$) after 3 months, while the reduction in the control group was not statistically significant ($p > 0.05$). No significant difference was found between the 2 groups. However, after 6 months, both the treatment and control groups showed significantly decreased DAS28-CRP and disease activity levels ($p < 0.001$ for the treatment group and $p < 0.05$ for the control group). In particular, when comparing the 2 groups after 6 months, the treatment group significantly differed from the control group in terms of DAS28-CRP ($p = 0.013$; $OR = 0.60$) and disease activity level ($p = 0.028$). The *OR*s for the disease activity

Table 2. Changes between the groups in the clinical parameters of periodontitis at baseline, and after 3 and 6 months of periodontal treatment

Parameter	Baseline				Assessment at 3 months				Assessment at 6 months			
	treatment group $n = 41$	control group $n = 41$	*p*-value	*OR* (95% *CI*)	treatment group $n = 41$	control group $n = 41$	*p*-value	*OR* (95% *CI*)	treatment group $n = 38$	control group $n = 38$	*p*-value	*OR* (95% *CI*)
PlI *Me (IQR)*	1.3 (0.8–1.6)	0.9 (0.8–1.7)	0.290[b]	1.41 (0.68–2.95)	0.6 (0.3–1.2)	1.0 (0.6–1.4)	0.064[b]	0.46 (0.19–1.08)	0.9 (0.3–1.3)	1.0 (0.4–1.3)	0.526[b]	0.80 (0.34–1.92)
GI *Me (IQR)*	1.1 (1.0–1.3)	1.0 (0.6–1.1)	0.008*[b]	6.45 (1.61–25.78)	0.3 (0.1–0.8)	1.0 (0.5–1.1)	<0.001*[b]	0.09 (0.03–0.29)	0.4 (0.1–0.8)	1.0 (0.7–1.1)	<0.001*[b]	0.06 (0.02–0.24)
%BOP [%] *Me (IQR)*	0.1 (0.1–0.2)	0.1 (0.0–0.1)	0.051[b]	14.58 (0.69–307.15)	0.0 (0.0–0.1)	0.1 (0.0– 0.1)	<0.001*[b]	0.0 (0.00–0.04)	0.0 (0.0–0.1)	0.1 (0.0–0.1)	0.056[b]	0.32 (0.01–13.72)
PPD [mm] *Me (IQR)*	1.4 (1.1–1.8)	1.1 (0.9–1.2)	0.016*[b]	2.90 (1.03–8.12)	0.9 (0.7–1.0)	1.2 (1.0–1.4)	<0.001*[b]	0.02 (0.00–0.16)	0.8 (0.7–0.9)	1.1 (0.9–1.4)	<0.001*[b]	0.02 (0.00–0.15)
Periodontal category												
non-PD	0 (0)	0 (0)		1	26 (63.4)	0 (0)			33 (86.8)	5 (13.2)		1
moderate PD	24 (58.5)	26 (63.4)	0.651[a]	1	15 (36.6)	28 (68.3)	<0.001*[a]	NA	5 (13.2)	24 (63.1)	<0.001*[a]	0.03
severe PD	17 (41.5)	15 (36.6)			0 (0)	13 (31.7)			0 (0)	9 (23.7)		(0.01–0.14)
n (%)												
CAL [mm] *Me (IQR)*	2.3 (1.7–2.5)	1.7 (1.4–2.5)	0.033*[b]	1.71 (0.93–3.17)	1.5 (1.2–1.9)	2.1 (1.6–2.6)	<0.001*[b]	0.16 (0.06–0.45)	1.5 (1.1–1.8)	1.9 (1.5–2.8)	0.001*[b]	0.23 (0.10–0.54)
CAL level												
<3 mm	0 (0)	0 (0)		1.22	0 (0)	0 (0)		6.22	0 (0)	0 (0)		7.83
3–5 mm	20 (48.8)	18 (43.9)	0.658[a]	(0.51–2.90)	36 (87.8)	22 (53.7)	0.001*[a]	(2.03–19.04)	36 (92.3)	23 (60.5)	0.001*[a]	(2.04–30.05)
>5 mm	21 (51.2)	23 (56.1)		1	5 (12.2)	19 (46.3)		1	3 (7.7)	15 (39.5)		1
n (%)									($n = 39$)			

PlI – plaque index; GI – gingival index; %BOP – percentage of sites with bleeding on probing; PPD – probing pocket depth; PD – periodontal disease; CAL – clinical attachment loss; NA – not applicable; [a] Fisher's test; [b] Wilcoxon test; * statistically significant. 95% *CI* was not applied in case of $OR = 1$.

Table 3. Changes between the groups in the clinical parameters of rheumatoid arthritis (RA) after 3 and 6 months of periodontal treatment

Parameter	Baseline				Assessment at 3 months				Assessment at 6 months			
	treatment group $n = 41$	control group $n = 41$	*p*-value	*OR* (95% *CI*)	treatment group $n = 41$	control group $n = 41$	*p*-value	*OR* (95% *CI*)	treatment group $n = 38$	control group $n = 38$	*p*-value	*OR* (95% *CI*)
DAS28-CRP *Me (IQR)*	4.2 (3.5–5.8)	4.1 (3.7–5.4)	0.461[b]	1.12 (0.83–1.52)	3.5 (3.0–4.7)	4.0 (3.5–4.7)	0.206[b]	0.80 (0.53–1.21)	3.2 (2.5–4.0)	3.6 (3.3–4.5)	0.013*[b]	0.60 (0.37–0.97)
RA disease activity level *n* (%)												
remission	1 (2.4)	2 (4.9)		1	9 (21.9)	2 (4.9)		1	12 (31.6)	3 (7.9)		1
low	3 (7.3)	5 (12.2)	0.756[a]	1.20 (0.07–19.63)	8 (19.5)	5 (12.2)	0.051[a]	0.36 (0.05–2.37)	7 (18.4)	5 (13.1)	0.028*[a]	0.35 (0.06–1.93)
moderate	22 (53.7)	22 (53.6)		2.00 (0.17–23.70)	17 (41.5)	28 (68.3)		0.13 (0.03–0.70)	18 (47.4)	28 (73.7)		0.16 (0.04–0.65)
high	15 (36.6)	12 (29.3)		2.50 (0.20–31.00)	7 (17.1)	6 (14.6)		0.26 (0.04–1.70)	1 (2.6)	2 (5.3)		0.13 (0.01–1.89)

DAS28-CRP – Disease Activity Score 28 based on C-reactive protein; [a] Fisher's test; [b] Wilcoxon test; * statistically significant. 95% *CI* was not applied in case of *OR* = 1.

level with regard to the treatment and control groups were 0.35, 0.16 and 0.13, respectively. Thus, the intensive periodontal treatment led to a significant reduction in the disease activity indices in patients with RA as compared to those receiving only basic treatment.

Changes in the immunohistochemical features of rheumatoid arthritis after 3 and 6 months of periodontal treatment

The comparison of the RA immunohistochemical features between the treatment and control groups after 3 and 6 months is presented in Table 4. Some patients were not able to complete immunohistochemical tests (missing data).

The results of periodontal treatment showed that the immunohistochemical indices decreased in both groups; these changes became more evident in the treatment group, but no statistically significant difference was found ($p > 0.05$). In particular, the RA patients who were treated for advanced periodontitis showed the ACPAs level (*Me*) decreasing from 156 to 42.7 ($p < 0.001$), while the ACPAs level (*Me*) in the control group decreased from 186 to 102.3 ($p = 0.032$). After 6 months, the treatment group showed significant reductions in serum ESR ($p < 0.001$), ACPAs ($p < 0.001$) and the ACPAs positive test ($p = 0.014$), while the control group showed a reduction only in the ACPAs concentration ($p = 0.032$). As the 2 groups were treated with the standard regimens performed at Cho Ray Hospital, the clinical and paraclinical parameters decreased,

Table 4. Changes between the groups in the immunohistochemical features of rheumatoid arthritis (RA) after 3 and 6 months of periodontal treatment

Parameter	Baseline				Assessment at 3 months				Assessment at 6 months			
	treatment group	control group	*p*-value	*OR* (95% *CI*)	treatment group	control group	*p*-value	*OR* (95% *CI*)	treatment group	control group	*p*-value	*OR* (95% *CI*)
CRP [mg/L] *Me (IQR)* $n = 78$	5.8 (1.5–13.0)	3.4 (0.9–8.1)	0.158[b]	1.01 (0.99–1.03)	3.9 (1.5–12.9)	4.4 (1.2–15.2)	0.969[b]	1.01 (0.98–1.03)	3.5 (0.7–9.9)	4.4 (1.1–11.8)	0.511[b]	0.99 (0.96–1.02)
RF [IU/mL] *Me (IQR)* $n = 74$	19.6 (8.0–96.7)	8.8 (8.0–96.0)	0.848[b]	1	8.0 (8.0–50.8)	8 (8.0–49.2)	0.867[b]	1.00 (0.99–1.00)	12.1 (8.0–65.9)	13.6 (7.9–57.0)	0.932[b]	1.00 (0.99–1.00)
RF level *n* (%)												
positive	18 (47.4)	14 (38.9)	0.462[a]	1.41 (0.56–3.56)	14 (36.8)	12 (33.3)	0.752[a]	1.17 (0.45–3.04)	17 (44.7)	16 (45.7)	0.933[a]	0.96 (0.38–2.42)
negative	20 (52.6)	22 (61.1)		1	24 (63.2)	24 (66.7)		1	21 (55.3)	19 (54.3)		1
ACPAs [IU/mL] *Me (IQR)* $n = 80$	156 (14.0–614.0)	186 (8.0–702.0)	0.889[b]	1	NA	NA	–	–	42.7 (7.1–185.0)	102.3 (9.6–794.0)	0.189[b]	1
ACPAs level *n* (%)												
positive	27 (69.2)	29 (70.7)	0.884[a]	0.93 (0.36–2.42)	NA	NA	–	–	23 (59.0)	25 (65.8)	0.537[a]	0.75 (0.30–1.89)
negative	12 (30.8)	12 (29.3)		1	NA	NA	–	–	16 (41.0)	13 (34.2)		1
ESR [mm/h] *Me (IQR)* $n = 73$	30 (21–65)	30.5 (15–51)	0.436[b]	1.01 (0.99–1.03)	28 (17–41)	24 (14.5–40.5)	0.633[b]	1.00 (0.98–1.02)	20.5 (11–28)	20.5 (11–32)	0.917[b]	1.00 (0.98–1.02)

Serum levels of: CRP – C-reactive protein; RF – rheumatoid factor; ACPAs – anti-citrullinated protein autoantibodies; and ESR – erythrocyte sedimentation rate; NA – not applicable; [a] Fisher's test; [b] Wilcoxon test; * statistically significant. 95% *CI* was not applied in case of *OR* = 1. Some patients were not able to complete immunohistochemical tests (missing data).

but the treatment group showed a more significant reduction than the control group. The *OR*s of RF, CRP and ACPAs between the treatment group and the control group were 0.96, 0.99 and 0.75, respectively. However, when comparing the 2 groups, this difference was not statistically significant. There was no reduction in the serum levels of RF and CRP during the treatment in either group.

Discussion

Clinical studies show different results regarding the role of non-surgical periodontal treatment in RA. Our study is the first to investigate comprehensively the effect of non-surgical periodontal treatment on the clinical characteristics and serum indices in Vietnamese patients with active RA and PD. The present RCT was performed on a large number of patients and with a long follow-up period (Table 5), with the full assessment of the RA condition, considering disease severity and immunohistochemical features. The study showed that after 6 months of treatment for RA and periodontitis, all RA clinical parameters decreased. Meanwhile, in the control group, only a few clinical parameters decreased, which is consistent with the results of Al-Katma et al.[26] and Okada et al.[27] In a study by Zhao et al., the authors divided patients into 4 groups: PD+/RA+; PD−/RA+; PD+/RA−; and PD−/RA−.[14] In the PD+/RA+ group, the levels of all RA markers (DAS28, high-sensitivity CRP – hsCRP, ESR, and ACAPs) decreased significantly after non-surgical periodontitis treatment within 1 month.[14]

Most of the studies report no decrease in the serum CRP levels after reassessment. The CRP level increases in systemic inflammatory diseases, such as RA, or infections, such as PD. In our study, there was a statistically significant reduction in the CRP levels in the treatment group, while in the control group, the reduction was not statistically significant, which is consistent with 2 other studies.[14,28] Addressing the inflammation of the periodontal tissue after non-surgical treatment can be very important for reducing the CRP levels. In their study evaluating the serum ACPAs levels, Okada et al. did not find any difference after treatment, which could potentially be attributed to the small sample size and the short follow-up period.[27] On the other hand, Lappin et al. found a slight difference.[29] However, this study had no control group and the participants were not RA patients.[29] In a study by Kaushal et al.,[30] the value of the ACCP index, when evaluated for each group, showed no significant difference before and after the intervention, which is similar to the results of Okada et al.[27] This may be due to the insufficient sample size or the mild severity of PD in the study group. However, the CRP levels in the treatment group at 2 months after treatment were significantly different from baseline.[30] Our study showed that after the combined treatment of PD and RA, ACPAs and the rate of ACPA positive tests significantly decreased ($p < 0.01$) (Table 5).

In their study, Cosgarea et al. assessed the impact of non-surgical treatment on the clinical periodontal indicators, the inflammation index and quantified periodontal bacteria in the gingival fluid of patients with PD

Table 5. Comparison of the results of other studies with regard to changes in the anti-citrullinated protein autoantibodies (ACPAs) after treatment

		Serum ACPAs level [IU/mL]					
		treatment group			control group		
		baseline	reassessment	*p*-value	baseline	reassessment	*p*-value
Okada et al.[27] 2013	control: *n* = 29 treatment: *n* = 26 (2 months) (range)	183.5–24.1	197.2–24.1	0.070	291.3–26.1	219.9–25.6	0.990
Lappin et al.[29] 2013	treatment: *n* = 2 (6 months) *Me* (*IQR*)	0.79 (0.37–6.11)	0.17 (0.00–12.68)	0.010*	NA	NA	–
Kaushal et al.[30] 2019	control: *n* = 20 treatment: *n* = 20 (2 months) *M* ±*SD*	161.78 ±12.71	161.89 ±12.73	0.140	153.18 ±16.05	153.39 ±16.09	0.346
Zhao et al.[14] 2018	treatment: *n* = 18 (1 month) *M* ±*SD*	102.24 ±97.70	57.46 ±47.96	<0.001*	NA	NA	–
Present study	control: *n* = 41 treatment: *n* = 41 (6 months) *Me* (*IQR*)	156 (14.0–614.0)	42.7 (7.1–185.0)	<0.001*	186 (8.0–702.0)	102.3 (9.6–794.0)	0.032*
	ACPAs (+) *n* (%)	27 (69.2)	23 (59.0)	0.014*	29 (70.7)	25 (65.8)	0.564
	ACPAs (−) *n* (%)	12 (30.8)	16 (41.0)		12 (29.3)	13 (34.2)	

NA – not applicable; * statistically significant.

and RA, in comparison with patients suffering from PD only.[28] Their results showed that in both groups there was improvement in the clinical periodontal indicators. In the RA group, DAS28, CRP and ESR were decreased; however, only the reduction in CRP was statistically significant at 3 months. The DAS28 change was positively correlated with the amount of *Porphyromonas gingivalis* and was inversely correlated with PlI. The authors concluded that non-surgical treatment helped to improve the periodontal status of PD patients, with or without RA. Non-surgical treatment not only improved the periodontal status, but also reduced CRP in the group with both PD and RA.[28] However, in a study by Mariette et al., the results showed that these interventions effectively reduced bacteria, but did not improve the status of RA.[31] In patients with ACPAs(+), such treatment was not effective for RA.[31] Our study showed that the treatment of RA in combination with non-surgical periodontal treatment improved the RA condition. After 6 months of advanced periodontal treatment, the *Me* value of DAS28-CRP decreased from 4.2 to 3.2. The effect on the treatment group was more significant as compared to the control group. After the intervention, the *Me* value of the serum ACPAs level decreased from 156 to 42.7. Furthermore, the rate of ACPAs positive test also decreased from 69.2% to 59%.

Limitations

The limitation of our study is that RA treatment methods may have affected the efficiency of PD treatment.[18,25,30] However, all RA patients had established RA (>12 months), and were treated with DMARDs, NSAIDs and corticosteroids. It is important to keep in mind that RA is a chronic condition, which requires from patients changing their medications upon the recommendations of doctors. Hence, it is difficult to obtain exactly homogeneous study groups. Moreover, the combination of local treatment (periodontal sites) and a systemic intervention (RA treatment) may decrease the RA severity score and the PD condition synergistically. Previous studies also failed to obtain RA patient groups that were treated identically.[27,30,32] Although we were not able to control RA treatment methods, our previous cross-sectional, descriptive study found that the incidence of periodontitis cases in the RA group was significantly higher than in the control group (67% and 28%, respectively), while severe and moderate periodontitis were common in the RA group.[8] Therefore, we suggested that interdisciplinary and simultaneous treatment for both diseases be necessary to ensure overall efficiency. Finally, we mentioned that by the end of the study, all patients were to receive advanced periodontal treatment, including supragingival scaling and root planing. There was no risk of participating in the study, as after finishing the study, the patients from the control group received the same non-surgical periodontal treatment as the study group.

Conclusions

In conclusion, our study provided comprehensive evidence of the effect of non-surgical periodontal treatment on the clinical characteristics and serum indices in patients with active RA and PD. Non-surgical periodontal treatment can help decrease RA severity by significantly reducing DAS28-CRP, disease activity classification, the serum ACPAs levels, and ESR in patients. Dentists and rheumatologists should provide care as well as the treatment and prevention of dental diseases for RA patients in order to achieve better outcomes for both RA and PD.

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Original papers

Effect of different surface treatment on the repair bond strength of feldspathic porcelain

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Abstract

Background. Considering the use of silane-containing universal adhesives to enhance the repair bond strength of porcelain restorations, a question arises whether the application of these adhesives eliminates the need for a separate application of silane or not.

Objectives. This study aimed to assess the effect of various kinds of surface treatment, including hydrofluoric acid (HF) etching, the application of bis-silane and the use of universal adhesives, on the repair bond strength of feldspathic porcelain.

Material and methods. In this experimental in vitro study, 70 porcelain disks were fabricated and divided into 7 groups ($n = 10$) for the following types of surface treatment: C (control group) – HF etching + silane + Porcelain Bond; HSB – HF etching + Single Bond; HSSB – HF etching + silane + Single Bond; HAB – HF etching + All Bond; HSAB – HF etching + silane + All Bond; HFB – HF etching + FuturaBond®; and HSFB – HF etching + silane + FuturaBond. After applying different kinds of surface treatment, the specimens were light-cured and the Filtek® Z250 composite was bonded to the treated surfaces. The specimens were incubated in distilled water at 37°C for 24 h, and then underwent 5,000 thermal cycles. The repair bond strength of porcelain was measured and the mode of failure was determined under a stereomicroscope. Data was analyzed using the one-way analysis of variance (ANOVA) and Tukey's test.

Results. Differences in the porcelain repair bond strength were significant among the groups ($p < 0.001$). Bond strength for Single Bond ($p < 0.001$) and All Bond ($p < 0.001$) along with silane was significantly higher than for the application of these adhesives without a separate silane application step. This difference was not significant for FuturaBond. Mixed failure was the dominant mode of failure in all groups.

Conclusions. The application of silane, irrespective of the use of universal adhesives with or without silane, increased the porcelain repair bond strength. Thus, a separate silane application step following HF etching and the use of universal adhesives with or without silane can enhance the repair bond strength of feldspathic porcelain.

Key words: dental adhesives, shear strength, dental porcelain, silanes, dental etching, hydrofluoric acid

Introduction

Glass-ceramics and feldspathic porcelains are 2 premier esthetic materials widely utilized to prepare full-ceramic or metal-ceramic crowns for custom veneer restorations in esthetic dentistry. These materials provide a biocompatible and corrosion-resistant veneering layer as well as an esthetic appearance.[1–3] Due to their excellent esthetic properties, they are suitable for ceramic laminate veneers, inlays and onlays.[4] However, porcelain restorations may fail in the oral environment due to occlusal forces, trauma, internal defects, or an inappropriate design.[3,5] Clinical studies have also reported the prevalence of fracture of ceramic restorations to be 5–10%, following 10 years of clinical service in the oral cavity.[6] Due to such factors as a high cost of fabrication of a new restoration, saving time and difficult retrieval of old restorations, the repair or reconstruction of ceramic or metal-based restorations has been suggested as a clinical solution.[5,7]

Restorative procedures include indirect methods (laboratory repairing techniques) and/or direct techniques, which use resin composite materials intraorally for repairing the fractured porcelain.[1,7,8] Current direct restoration methods involve micromechanical and chemical bonding as well as some treatment for preparing the ceramic surface, which affects the resin–porcelain bonding.[3] Ceramic surfaces can be prepared by means of several methods, including acid etching with hydrofluoric acid (HF),[9,10] air abrasion with AL_2O_3 particles,[9,11] silica coating,[3] and laser irradiation.[12,13] These procedures are applied to increase surface roughness and improve bond strength.[3,14] Silane coupling components are utilized not only to improve the wettability of the ceramic surface, but also to chemically strengthen the ceramic–composite resin bond.[3,9] Silane bilaterally attaches to the silicon dioxide groups and methacrylate groups, bonding the ceramic surface to the resin.[9,15] Therefore, silane makes the ceramic surface both organophilic and hydrophobic, which plays a pivotal role in establishing the ceramic–resin chemical bond.[3]

Recently, a new generation of bonding agents, known as universal adhesives, has been introduced. They provide a durable, long-term bond to different substrates, such as zirconia, silica-based ceramics, non-silica-based ceramics, noble and non-noble metals, and stainless steel.[16]

Objectives

This study aimed to assess the effect of different kinds of surface treatment, including HF etching, the application of a two-part silane coupling agent and the use of universal adhesives, on the repair bond strength of feldspathic porcelain in vitro. The null hypothesis was that different surface treatment methods do not affect the strength of the repair bond of the composite resin to ceramic blocks.

Material and methods

This experimental, in vitro study evaluated 70 feldspathic porcelain disks measuring 6 mm × 2 mm (Ceramco®3; Dentsply, York, USA). The specimens were inspected under a magnifier at ×10 magnification to ensure the absence of microcracks. For the standardization of porcelain surfaces, they were polished with 400- and 600-grit silicon carbide disks under running water for 15 s. The specimens were rinsed with water and dried. After that, they were cleaned with phosphoric acid, and then 9.5% HF (Porcelain Etchant gel; Bisco, Schaumburg, USA) was applied on the surfaces of the specimens for 2 min. The specimens were then rinsed with water for 1 min and dried with air spray for 1 min. Next, they were subjected to the following interventions:

- group C (control): One layer of a two-part silane coupling agent (Bis-Silane Parts A & B; Bisco) was applied on the surfaces of the specimens with a microbrush for 1 min and dried with air spray for 30 s. Next, 1 layer of porcelain bonding resin (Porcelain Bond; Bisco) was applied on the surfaces of the specimens and light-cured for 20 s with a light-curing unit (Demetron® Optilux 401 device; Kerr, Danbury, USA) at a light intensity of 600 mW/cm^2;
- group HSB: One layer of the silane-containing Single Bond Universal adhesive (3M ESPE, St. Paul, USA) was applied on the surfaces of the specimens in this group. The bonding agent was gently rubbed on the surface for 15 s with an applicator and thinned with gentle air spray for 5 s to evaporate the solvent. The specimens were light-cured for 20 s as described for group C;
- group HSSB: One layer of bis-silane (Bis-Silane Parts A & B; Bisco) was applied on the surfaces of the specimens with a microbrush for 1 min. The silanized surfaces were then dried with air spray for 30 s. Next, 1 layer of the silane-containing Single Bond Universal adhesive (3M ESPE) was applied on the surfaces as explained for group HSSB and light-cured for 20 s as described for the previous groups;
- group HAB: One layer of the All Bond Universal bonding agent (Bisco) was applied on the surfaces of the specimens and light-cured for 20 s as described for the previous groups;
- group HSAB: One layer of bis-silane (Bis-Silane Parts A & B; Bisco) along with 1 layer of All Bond Universal (Bisco) were applied on the surfaces of the specimens and light-cured for 20 s as described for the previous groups;
- group HFB: One layer of the FuturaBond® Universal bonding agent (Voco, Cuxhaven, Germany) was applied on the surfaces of the specimens and light-cured for 20 s as described for the previous groups;
- group HSFB: One layer of bis-silane (Bis-Silane Parts A & B; Bisco) along with 1 layer of FuturaBond Universal (Voco) were applied on the surfaces of the specimens and light-cured for 20 s as described for the previous groups.

The A1 shade of the Filtek® Z250 nanofilled composite (3M ESPE) at a thickness of 2 mm was applied into transparent, cylindrical Tygon® tubes measuring 3 mm × 4 mm. The tubes were placed on the bonding surface. Each increment was light-cured for 40 s. Composite cylinders were additionally cured for 120 s from the porcelain surface in all 3 dimensions at an angle of 45°. Next, the Tygon tubes were separated and the specimens were inspected under a stereomicroscope (Nikon, Tokyo, Japan) at ×10 magnification. Then, the specimens were immersed in distilled water and placed in an incubator (Pars Azma Co., Tehran, Iran) at 37°C for 24 h. Next, the specimens underwent 5,000 thermal cycles at 5–55°C with a dwell time of 30 s and a transfer time of 10 s in a thermocycler (TC-300; Vafaei Industrial, Tehran, Iran). The specimens were then mounted in auto-polymerizing acrylic resin (Acropars; Marlic Co., Tehran, Iran) and transferred to a universal testing machine (Z050; ZwickRoell, Ulm, Germany) to measure shear bond strength (SBS). The load was applied to the composite–porcelain interface at a crosshead speed of 1 mm/min until debonding occurred. The load at debonding was recorded and bond strength was calculated in megapascals (MPa).

The composition of the materials used in the study is presented in Table 1.

The mode of failure of the specimens was determined under a stereomicroscope (Nikon) at ×20 magnification. The mode of failure was categorized as adhesive, cohesive or mixed (adhesive/cohesive). One specimen from each category of failure was inspected under a scanning electron microscope (Nikon) at ×10, ×50 and ×100 magnification to further analyze the failure mode in detail.

Statistical analysis

Data was analyzed using IBM SPSS Statistics for Windows, v. 21.0 (IBM Corp., Armonk, USA). The mean (*M*), standard deviation (*SD*), standard error (*SE*), and upper and lower bounds with 95% confidence intervals (*CI*s) of the repair bond strength of feldspathic porcelain in different surface treatment groups were calculated and reported. First, the normal distribution of data was evaluated using the Kolmogorov–Smirnov test. Since data was normally distributed, the effect of the application of bis-silane and a universal adhesive on the repair bond strength of porcelain was separately analyzed using the two-way analysis of variance (ANOVA). The repair SBS of feldspathic porcelain in different groups was compared using one-way ANOVA. Since the result of one-way ANOVA was significant, pairwise comparisons were performed using Tukey's multiple comparison test. The level of significance was set at 0.05 ($\alpha = 0.05$).

Results

Table 2 presents the mean values of the repair SBS of feldspathic porcelain in different surface treatment groups.

According to the results of one-way ANOVA, significant differences were found in the repair bond strength of feldspathic porcelain between different surface treatment groups ($p < 0.001$). In other words, the application of HF, universal adhesives and silane had significantly different effects on the repair bond strength of feldspathic porcelain.

Table 1. Characteristics of the selected studies

Material	Manufacturer	Components
Porcelain Etchant	Bisco, Shaumburg, USA	9.5% HA
Bis-silane	Bisco, Shaumburg, USA	ethanol, silane coupling agent, water
Porcelain bonding resin	Bisco, Shaumburg, USA	Bis-GMA, TEGDMA, UDMA
Single Bond Universal	3M ESPE, St. Paul, USA	HEMA, dimethacrylate, MDP monomer, silane methacrylate-modified polyalkenoic acid copolymer, ethanol, water, initiators, Vitrebond™ copolymer
All Bond Universal	Bisco, Shaumburg, USA	10-MDP dimethacrylate resin, HEMA, ethanol, water, initiators
FuturaBond® Universal	Voco, Cuxhaven, Germany	modified 10-MDP dimethacrylate resin, HEMA, ethanol, water, carboxylic acid ester, initiators
Filtek® Z250 composite resin	3M ESPE, St. Paul, USA	Bis-GMA, Bis-EMA, TEGDMA, zirconia/silica fillers filler volume: 60% filler size: 0.01–3.5 µ

HA – hydrofluoric acid; Bis-GMA – bisphenol A-glycidyl methacrylate; TEGDMA – triethylene glycol dimethacrylate; UDMA – urethane dimethacrylate; HEMA – hydroxyethyl methacrylate; MDP – methacryloyloxydecyl dihydrogen phosphate; Bis-EMA – ethoxylated bisphenol A-glycidyl methacrylate.

Table 2. Repair shear bond strength (SBS) [MPa] of feldspathic porcelain for surface treatment with universal adhesives, silane and hydrofluoric acid (HF)

Group	SBS [MPa]	Min	Max
C[a]	13.31 ±1.16	11.22	15.23
HSB[b]	11.39 ±1.21	9.80	13.70
HSSB[a]	14.08 ±1.39	11.27	16.11
HAB[b]	11.21 ±0.71	10.10	12.26
HSAB[a]	13.92 ±1.62	11.27	16.80
HFB[a,b]	12.27 ±1.59	9.80	14.80
HSFB[a]	13.66 ±1.61	11.37	16.66

Groups: C (control group) – HF + bis-silane + Porcelain Bond;
HSB – HF + Single Bond; HSSB – HF + bis-silane + Single Bond;
HAB – HF + All Bond; HSAB – HF + bis-silane + All Bond;
HFB – HF + FuturaBond®; HSFB – HF + bis-silane + FuturaBond®;
min – minimum; max – maximum; different superscript letters indicate statistically significant differences between various surface treatment methods ($p < 0.05$).
Data presented as mean (M) ± standard deviation (SD).

Two-way ANOVA revealed that the effect of an additional silane application step on the repair bond strength of porcelain was significant ($p < 0.0001$). However, the effect of the type of universal adhesive ($p = 0.660$) as well as the interaction effect of the type of universal adhesive and an additional bis-silane application step ($p = 0.240$) on the repair bond strength of feldspathic porcelain were not significant. In other words, the application of bis-silane increased the repair bond strength irrespective of the type of universal adhesive, with or without silane in its composition.

The current findings revealed significant differences in the repair bond strength of feldspathic porcelain following the application of bis-silane and universal adhesives with or without silane. The maximum repair bond strength of porcelain was noted following the application of HF along with bis-silane and Single Bond containing silane (14.08 MPa). The minimum bond strength was noted following HF etching along with the application of the All Bond adhesive without silane without the additional application of bis-silane (11.21 MPa).

Pairwise comparisons revealed that the bond strength following the application of HF + bis-silane + Single Bond containing silane was significantly higher than the bond strength following the application of HF + Single Bond containing silane without a separate application of bis-silane ($p < 0.001$). On the other hand, the bond strength following the application of HF + bis-silane + All Bond was significantly higher than that following the application of HF + All Bond without silane ($p < 0.001$). The porcelain repair bond strength following the application of HF + bis-silane + FuturaBond was higher than that following the application of HF + FuturaBond without silane; however, this difference did not reach statistical significance ($p > 0.05$). In all groups, the application of bis-silane increased the porcelain repair bond strength irrespective of the presence or absence of silane in the composition of the universal adhesive used. However, in the case of FuturaBond, which does not contain silane, the application of bis-silane could not significantly increase bond strength.

Table 3 shows different modes of failure in different surface treatment groups. No case of adhesive failure was noted in any group, and the mixed failure was the dominant mode of failure in all groups. Cohesive failure was noted only in a few specimens.

Table 3. Modes of failure in different surface treatment groups ($n = 10$)

Group	Mode of failure [%]			
	cohesive	mixed	adhesive	total
C	20	80	–	100
HSB	–	100	–	100
HSSB	20	80	–	100
HAB	–	100	–	100
HSAB	30	70	–	100
HFB	–	100	–	100
HSFB	10	90	–	100
Total	11.4	88.6	–	100

Discussion

This study evaluates the effect of different kinds of surface treatment, including HF etching, bis-silane and universal adhesives, on the repair bond strength of feldspathic porcelain in vitro. Considering the results, the null hypothesis that different surface treatment methods do not affect the repair bond strength of the composite to ceramic blocks was rejected.

Adhesive dentistry have recently enabled the use of adhesives for the repair of ceramic restorations instead of their replacement.[16] In the present study, Porcelain Bond along with bis-silane and HF conditioning was used for surface treatment in the control group. The porcelain repair bond strength values in the experimental groups were compared with the value in the control group. This surface treatment modality is commonly used for conditioning the porcelain surface prior to porcelain repair.[14] On the other hand, Filtek Z250 was used to enable the comparison of the results. Accordingly, the HF etching and silanization methods actually improved the repair bond strength of feldspathic porcelain.[15] Etching porcelain with HF selectively dissolves the glassy phase, resulting in irregular microporosity and an increased surface area, and creating a micromechanical interlock, which enhances the penetration of restorative materials into tiny undercuts in the etched porcelain surface and reinforces the formation of hydroxyl groups on the porcelain surface.[9,17] The etching of the ceramic surface results in the formation of volatile silicon tetrafluoride, which creates a soluble ionic complex (hexafluorosilicate) with HF. The molecules in the chemical composition of the etched surface can bond to proton to form tetrafluorosilicic acid.[18] Dental clinicians should be well aware of the serious risk of exposure of oral tissues to HF, especially in cases when rubber dam isolation cannot be performed.

However, the gel-etching of glass-ceramics is still considered the golden standard method. This conditioning medium is considered advantageous as compared to the air-abrasion method, which leaves microcracks in the ceramic.[10]

After applying the gel, the organic hydrolyzed functional groups react with the surface hydroxyl groups present in the inorganic substrate and create a siloxane bond (Si–O–Si), producing a water molecule by-product. The organic non-hydrolyzed functional groups have carbon–carbon double bonds and can be polymerized with composite resin monomers, which contain double bonds.[9,18]

It seems that there exists an equilibrium between the number of the exposed hydroxyl groups present in the inorganic substrate and the hydrolyzed functional groups present in silane; the quality of the siloxane bond can be predicted based on the concentration of the silane solution.[18]

The surface treatment protocol also plays a role in this regard by quantifying the number of the exposed hydroxyl groups.

Silane can bond to silica fillers as well as react with organic materials, such as composite resin, and inorganic materials, such as porcelain, via its functional groups and dual reactions, and enhance their surface wettability as such.[15] The present study indicated that a separate bis-silane application step increased the repair bond strength of feldspathic porcelain in all groups.

To reduce the clinical stages of the adhesive procedures, manufacturers have incorporated a silane coupling agent in the composition of some universal adhesives, claiming that there is no extra need for a separate ceramic primer for chemical bonding to a glass-ceramic[19] (among the materials used in the present study, Single Bond contains silane in its composition, while All Bond and FuturaBond are devoid of it). The results of the present study showed that the additional step of bis-silane application along with the use of universal adhesives with or without silane in their composition significantly increased the repair bond strength of feldspathic porcelain. Although in the case of using universal adhesives with or without silane the repair bond strength of porcelain was statistically lower than that following the use of universal adhesives along with a separate application of bis-silane, this difference did not seem to be clinically significant. However, considering the significance of high repair bond strength of porcelain, the additional step of silane application should not be eliminated so that the maximum bond strength could be achieved.

Our results are in agreement with those reported by Zaghloul et al.,[20] Kim et al.,[21] Kalavacharla et al.,[22] Vasconcelos Moro et al.,[23] AlRabiah et al.,[24] Suárez-Moya et al.,[14] and Yao et al.,[25] who claimed that a separate application of silane along with HF etching and the application of a universal adhesive significantly increased the repair bond strength of feldspathic porcelain.

Universal adhesives show decreased performance when they are used as ceramic primers, which is suggested, as the silane coupling agent is less stable in the water acidic adhesive solution. The silanol groups (–Si–OH), produced from hydrolyzing the silane groups (–Si–CH_3) of the silane coupling agent in the presence of water, can adsorb and chemically bond to the glass.[15,19] Still, if the silanol groups undergo dehydroxylation after hydrolysis, they condense and form a siloxane $(–O–Si-O)_n$ oligomer, which does not adsorb to glass. The coupling capacity of the silane content to silica can be affected by any interaction between the different monomers of the universal adhesive, such as the presence of bisphenol A-glycidyl methacrylate (Bis-GMA), which prevents the silanol group and the substrate from the condensation reaction.[19]

Furthermore, some functional components of universal adhesives other than methacryloyloxydecyl dihydrogen phosphate (MDP), such as dimethacrylates and other additives, are reported to be affect bond strength.[19] These adhesive components improve the mechanical and bonding properties of the polymer by making it hydrophobic and reducing the ceramic–resin contact angle. This reduction leads to a closer interaction, which strengthens the bond.[19]

Functional monomers, cross-linking monomers, solvents, inhibitors, and activators may be present in the composition of universal adhesives in variable amounts and may affect bond strength as well. Undoubtedly, the amounts of monomers, diluting agents and the filler volume of different products are different depending on their manufacturing technology. Also, limited information is available regarding the shrinkage and strength of these adhesives, which can affect their physical and mechanical properties. Other influential factors in this respect include the application technique, differences between operators and inadequate time for drying the primer, which can affect bond strength following the application of universal adhesives on different substrates.

One surface pre-treatment method recognized as effective in enhancing the bond strength of surface micromechanical interlocking junctions and improving their adhesiveness is the erbium-doped yttrium aluminum garnet (Er:YAG) laser irradiation.[26] However, the controversial results of the Er:YAG laser application in ceramic surface pre-treatment, such as those reported by Akyıl et al.[27] and Shirinzad et al.,[28] where the Er:YAG laser provided lower bond strength, different power settings and ceramic materials should be considered for the clinical use of the Er:YAG laser irradiation. For example, while high power settings may cause crystal or matric destruction and create a heat-damaged layer, low power settings have a poor impact on the ceramic surface.[13] Most researches deduce that a water layer is created on the ceramic surface with a water-spraying device, causing the destruction of the local crystals or matrix of the ceramic due to high temperature or pressure during laser irradiation. Accordingly, the dispersed pits formed on the ceramic surface alter the mechanical retention and affect the bond strength of the repaired ceramic.[13] Therefore, setting the most suitable power is essential for the practical use of the Er:YAG laser.

However, more studies are required to establish the efficiency of the Er:YAG laser, as, for example, a study by Hou et al. showed that the Er:YAG laser could not be applied for all types of computer-aided design/computer-aided manufacturing (CAD/CAM) ceramics.[13] On the other hand, there are confirmative studies that suggest complementary treatment to be beneficial for increasing the efficiency of laser irradiation. A considerable instance is a report by Ahrari et al.,[12] suggesting the use of HF after laser irradiation in order to provide more efficient bond strength between the resin composite and feldspathic porcelain.

The current results showed that mixed failure was the dominant mode of failure in all groups and no case of adhesive failure was noted in any group. Cohesive failure ranked second after mixed failure, but with a much lower frequency; however, both were usually correlated with high values of SBS. The mode of failure is a criterion to determine the success of adhesive restorations. In this respect, adhesive failure is unacceptable, while mixed failure is acceptable and cohesive failure indicates ideal restorations.[29] In a study by de Melo et al., porcelain with a lesser crystalline phase and a greater glassy phase was shown to cause more cohesive failure.[30]

In the present study, the specimens underwent thermocycling before SBS assessment to test the durability of adhesives and simulate intraoral aging. Thermal cycles cause hydrolytic and thermal reductions, and by sudden thermal alterations, they simulate thermal degradation and decrease bond strength.[17] The dissimilar coefficients of the resin-based polymer and the thermal expansion of porcelain are possibly responsible for decreasing the bond strength at the interface of 2 materials during the alternation series of compression and expansion.[17]

The SBS test was performed in this study, as it enables the assessment of different substrates and has high reliability. On the other hand, anterior teeth are primarily subjected to shear loads and this test enables the comparison of bond strength values; thus, it was selected for the present study.

Also, despite numerous laboratory studies, there is insufficient information for predicting the clinical performance of the repair systems. Hence, further studies are required to determine the repair bond strength of zirconia. In addition, clinical studies are needed to confirm the results of the in vitro tests.

Conclusions

Considering the current results, a separate silanization procedure during porcelain surface treatment led to a significant increase in the repair bond strength of porcelain irrespective of the presence or absence of silane in the composition of universal adhesives. Therefore, a separate application of silane along with HF etching and the application of a universal adhesive for porcelain surface treatment can improve the repair bond strength of porcelain.

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Reviews

Demographic, clinical, laboratory, and genetic risk factors associated with COVID-19 severity in adults: A narrative review

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Abstract

Since the first report on it in December 2019 in Wuhan, China, the novel coronavirus disease 2019 (COVID-19) has rapidly spread throughout the world. Due to the lack of effective therapy available for COVID-19 patients, the identification of risk factors for the severe course of the disease is a matter of urgency. Therefore, the aim of this review was to report on evidence-based risk factors affecting the severity and prognosis of COVID-19. We searched the PubMed database for current literature to identify relevant publications concerning risk factors for COVID-19 severity. Demographic and social factors (age, gender, race, in-center communities/nursing homes), clinical factors (smoking, hypertension, obesity, diabetes, chronic lung diseases, cardiovascular diseases – CVD, chronic kidney disease – CKD, malignancies, dementia, cardiomyopathies, immunocompromised state), laboratory markers (C-reactive protein – CRP, leukocytosis, ferritin, interleukin (IL)-6, D-dimer, lactate dehydrogenase – LDH, aspartate aminotransferase – AST, procalcitonin, creatinine, lymphopenia, IL-2, IL-7, IL-10, granulocyte colony-stimulating factor – G-CSF, also known as colony-stimulating factor 3 – CSF 3, interferon gamma-inducible protein-10 – IP-10, monocyte chemoattractant protein-1 – MCP-1, macrophage inflammatory protein-1alpha – MIP1A, tumor necrosis factor alpha – TNF-α), and genetic factors related to both the virus and the host were discussed. The identification of the potential risk factors affecting the severity and prognosis of COVID-19 may provide a chance for earlier and more effective management of COVID-19.

Key words: risk factors, severity, demographics, COVID-19, SARS-CoV-2

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Introduction

Coronavirus disease 2019 (COVID-19) is a viral disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Currently, the outbreak of the COVID-19 pandemic has become a major clinical threat all over the world. The World Health Organization (WHO) declared the outbreak of COVID-19 as a worldwide pandemic in March 2020. Globally, as of November 19, 2020, there have been 55,928,327 confirmed cases of COVID-19, including 1,344,003 deaths, reported to WHO.[1] Based on genomic and phylogenetic studies, SARS-CoV-2 might originate from bat coronaviruses, although its exact origin remains unknown.[2–4]

The novel coronavirus is believed to be contagious during its incubation period, commonly observed as 3–7 days and being reported as up to 14 days.[5] The transmission of SARS-CoV-2 from patients who have not yet developed symptoms has been reported in numerous studies.[6–8] Coronavirus disease 2019 presents a wide variety of clinical manifestations. The most common symptoms of the disease which have been reported repeatedly include fever, cough, fatigue, muscle pain, headache, and dyspnea. Some other symptoms, such as diarrhea, expectoration, loss of taste and/or smell, hemoptysis, nasal obstruction, or runny nose, have been reported less frequently.[9,10] Recently, the immune system has been recognized to play a critical role in providing preexisting immunity to SARS-CoV-2.[11–13] Lymphopenia has been commonly observed in COVID-19, with levels more profound in more severe cases,[14,15] while peripheral bilateral ground-glass opacity or consolidation in chest computed tomography (CT) scans have been reported as the most common radiological finding.[16]

The spectrum of COVID-19 symptoms ranges from asymptomatic and mild symptomatic cases to a severe hyperinflammatory state, followed by acute respiratory distress syndrome (ARDS) and death.[14,17] The severity of the disease has been categorized as a mild, moderate, severe, and critical condition.[18,19] Fortunately, up to 40% of SARS-CoV-2 infections may be asymptomatic, suggesting that some people could be protected from the disease.[20] More than 80% of the infected patients present no more than mild symptoms, resembling a common cold with a dry cough, fatigue and fever.[10,21] In mild cases, dyspnea, the clinical evidence of pneumonia and respiratory insufficiency have not been observed, while resting blood oxygen saturation (SpO_2) remained usually above 93%.[22,23] Patients with moderate disease suffer from fever, respiratory symptoms or pneumonia. In severe disease, dyspnea and hypoxemia occur, with the respiratory rate reaching 30 breaths/min or more and $SpO_2 \leq 93\%$ under resting conditions.[24] In the most severe COVID-19 cases, ARDS, multiple organ dysfunction syndrome (MODS), shock, and coagulation abnormalities have been observed.[25–27] Severe cases of COVID-19 presenting with respiratory failure require early and prolonged support with mechanical ventilation.[28]

Risk factors for COVID-19 severity are complex and comprise demographic, clinical, laboratory, and genetic factors, concerning both the host and the virus variability. Until now, apart from symptomatic treatment, there is no evidence known for the effective causative therapy of COVID-19.[29,30] Therefore, identifying the main risk factors affecting the severity and prognosis of COVID-19 seems to be of particular importance. Hence, the aim of this narrative review was to report on evidence-based risk factors affecting the severity and prognosis of COVID-19.

Methods

The authors searched the PubMed database to identify relevant publications published between January 2020 and November 2020, and presenting demographic, clinical, laboratory, and genetic COVID-19 risk factors. The following inclusion criteria were used: original and review articles that discussed risk factors for the severity and outcome of COVID-19; the availability of the full text of the article; and papers published in the English language.

Demographic, social and clinical factors

Numerous studies have demonstrated that elderly patients are more likely to experience severe COVID-19 manifestation than their younger counterparts.[31] Older age (>65 years), male gender and obesity have been stated as the most common risk factors,[32,33] with age reported so far as the strongest determinant of the severity of the disease.[9,34,35] Also, social risk factors, typically inconducive to social distancing, have been reported to be of great importance. Thus, the COVID-19 pandemic has affected a large proportion of nursing home residents, with higher prevalence in the in-center dialysis community.[35,36] The Epidemiology Working Group for NCIP Epidemic Response has demonstrated comorbidities to be an important risk factor as well.[34] Cardiovascular diseases (CVD), arterial hypertension (HTA), diabetes,[37–39] chronic lung diseases,[40–42] severe asthma,[43] neoplasms,[44,45] and chronic kidney disease (CKD), especially under dialysis treatment, have been reported to increase the risk of COVID-19 severity and morbidity.[32,35,46–48] It has been recently reported that even a younger age of ≥60 years is associated with a major risk.[31,49] It has been calculated that the risk of COVID-19 unsuccessful outcome in people aged >80 years is increased more than twenty-fold when compared to 50–59-year-old individuals.[43] Presumably, the age-related diminishing of the physiological activity of both respiratory and cardiovascular systems provides a good explanation for the impaired clearance of pathogens.[50,51] Taking into account the dysfunction of arterial endothelium, nitric oxide (NO) availability – the main intracellular antiviral defense – decreases significantly in the elderly.[52] Zhou et al. showed that older age, the initial Sequential Organ Failure Assessment (SOFA) score >2, D-dimer >1 μg/1 mL, and the respiratory rate >24 breaths/min were independent risk factors for COVID-19 mortality in the Chinese population.[53] Also dementia was described as associated with a higher mortality rate in a study of 16,749 patients hospitalized for COVID-19.[54] Among symptoms, dyspnea has been recognized as an independent risk factor for the severity of the disease. Furthermore, a significant association between COVID-19 severity and the smoking status has been demonstated.[48,55]

Another significant risk factor for COVID-19 severity which has been noted is race. Black, South Asian and minority ethnic groups have been reported to be at higher risk of the poor outcome of COVID-19 as compared to Caucasians, even after the adjustment for other factors.[43,56,57]

In an observation of 1,289 oncological patients with solid tumors, age and the use of corticosteroids before COVID-19 diagnosis, together with thoracic primary tumor site, were independently associated with COVID-19 severity.[58] Except cytotoxic chemotherapy, associated with a slight increase in the risk of death, none of the anticancer drug protocols administered during the 3 months preceding COVID-19 had a significant effect on its mortality or severity. It is worth emphasizing that 39% of patients had their systemic anticancer treatment interrupted or stopped following COVID-19 diagnosis.[58]

According to the Center for Disease Control and Prevention (CDC), the most important risk factors for COVID-19 severity are cancer, CKD, chronic obstructive pulmonary disease (COPD), heart failure, coronary artery disease, cardiomyopathies, immunocompromised state from solid-organ transplant, obesity, Down syndrome, pregnancy, sickle-cell disease, smoking, and type 2 diabetes.[59] Regarding the pathophysiological importance of endocrine-immune-vascular interactions for the clinical course of COVID-19, metabolic syndrome has also been suspected to increase the risk of the severe course of COVID-19.[60] However, some data is inconsistent, which might be caused by the limited samples size or the presence of multiple confounding factors.[61–63] In the Chinese population, hypertension, diabetes and CVD were independently associated with COVID-19 severity, which was confirmed in a recent meta-analysis.[64] Despite initial controversies at the early stage of the pandemic, the use of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II type 1 receptor blockers (ARBs) could contribute to the improvement of COVID-19 outcome in hypertensive patients.[65] Recently, a prospective observational cohort study based on the data collected in the Risk stratification in COVID-19 patients in the ICU (RISC-19-ICU) registry showed that the main mortality predictors in critically ill COVID-19 patients were oxygenation deficit, and renal and microvascular dysfunction, together with coagulatory activation.[66]

The severity of COVID-19 might also be related to the amount of viral load, which has been observed to be higher in patients with severe COVID-19 than those with mild COVID-19.[67]

Laboratory risk factors

The monitoring of laboratory markers serves as a sensitive indicator of the severity of the disease, important for clinicians. Elevated levels of C-reactive protein (CRP), ferritin, interleukin (IL)-6, and plasma D-dimer are usually observed during infection. Hypercoagulability marked as elevated D-dimer concentration has been described to be indicative of poor COVID-19 prognosis.[68,69] Recently, IL-6 has been identified as the key predictor of mortality in COVID-19.[53] As SARS-CoV-2 originates from China, most of former studies regarding COVID-19 severity refer to the Chinese population. Huang et al. showed higher plasma levels of IL-2, IL-7, IL-10, granulocyte colony-stimulating factor (G-CSF), interferon gamma-inducible protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1alpha (MIP1A), and tumor necrosis factor alpha (TNF-α) in COVID-19 intensive care units (ICU) patients.[10] It has also been demonstrated that leukocytosis, high scores on the modified quick SOFA, an elevated serum level of aspartate aminotransferase (AST) (more than 3 times), and a serum level of creatinine of 2 mg/dL or more are markers of a significantly higher risk of ICU admission and mortality in COVID-19 patients.[70] The neutrophil-to-lymphocyte ratio has also been reported to be one of the major predictors of COVID-19 severity.[71]

A recent meta-analysis confirmed that reduced levels of lymphocytes and hemoglobin, elevated leukocytosis, AST, alanine aminotransferase (ALT), blood creatinine, blood urea nitrogen, high-sensitivity troponin, creatine kinase, high-sensitivity CRP, IL-6, D-dimer, ferritin, lactate dehydrogenase (LDH), and procalcitonin, and a high erythrocyte sedimentation rate were risk factors for severe COVID-19.[72] In the Chinese population, total protein and albumin concentrations have also been identified as independent risk factors for severe disease.[48] Lactate dehydrogenase and prealbumin have been demonstrated to be associated with the severity of the disease. The mortality risk is associated with age, LDH, CRP, D-dimer, and lymphopenia in patients with comorbidities.[73,74] An independent association between glycated hemoglobin (HbA_{1c}) and COVID-19 death rate has been also revealed.[75–77] Based on the secure health analytics platform which covers 40% of all patients in England (OpenSAFELY), it has been shown that a HbA_{1c} level of at least 58 mmol/mol is associated with a higher risk of COVID-19-related death.[43] Recently, IP-10 and MCP-1 have been demonstrated to be novel biomarkers of COVID-19 severity, which may be related to the risk of patients' death from COVID-19.[78]

Genetic factors

SARS-CoV-2 genetic diversity

Coronaviruses are RNA viruses that belong to order Nidovirales, family Coronaviridae and subfamily Coronavirinae.[79] The genome of one strain of SARS-CoV-2 is 29.9 kilo-bases (kb) in size, with 29,891 nucleotides[80] encoding 9,860 amino acids.[81] There are 2 types of SARS-CoV-2 (L and S), which are defined by means of 2 different single nucleotide polymorphisms (SNPs).[82] In a study comparing SARS-CoV-2 genetic diversity in mild and severe cases, it was demonstrated that the consensus sequences of all viruses were very similar, showing more than 99.8% sequence identity, regardless of the severity of the disease.[83] However, patients with severe symptoms exhibited a significantly higher number of variants in coding and non-coding regions as compared to mild cases, and it was concluded that within-host diversity might play a role in the development of severe disease outcomes in COVID-19 patients, particularly among the older ones.[83] Shikov et al. demonstrated that several rare *ACE2* variants, including rs146598386, rs73195521, rs755766792, and others, were likely to affect the outcome of COVID-19.[84] Among the accessory SARS-CoV-2 proteins, ORF3a is the largest one, containing 274 amino acids.[85] The potential role of ORF3a mutations in an elevated mortality rate for the SARS-CoV-2 infection through host immune evasion and immoderate cytokine storm has been recently shown. Majumdar and Niyogi revealed a decent association between SARS-CoV-2 ORF3a mutations and a higher mortality rate.[86]

Human genetic diversity

Recently, ABO blood groups have been implicated in susceptibility to the SARS-CoV-2 infection. Blood group A has been found to be associated with a minimally increased risk of acquiring COVID-19 in comparison with non-A groups; moreover, blood group O has been associated with a minimally decreased risk of acquiring COVID-19 in comparison with non-O groups.[25,87,88] The Rh(D) positive blood type was associated with the SARS-CoV-2 infection and death.[89]

The renin-angiotensin-aldosterone system (RAAS) is of great importance in COVID-19,[90] having considered the fact that angiotensin-converting enzyme 2 (ACE2) is the major receptor for SARS-CoV-2 on alveolar epithelial cells.[91] Transmembrane protease serine 2 (TMPRSS2) – a molecule that is necessary for spike protein (S protein) priming – and ACE2 are expressed, except alveoli, also in blood vessels, olfactory epithelium, the brain, the heart, the kidneys, the bladder, and the intestine, thus explaining the varied symptoms observed in COVID-19 patients.[92,93] Together with ACE2, TMPRSS2 and dipeptidyl peptidase-4 (DPP4) have been reported to play an important role in the severity of the disease.[94] It has been established that SARS-CoV-2 uses the receptor ACE2 for entry and TMPRSS2 for S protein priming.[91] The *ACE2* or *TMPRSS2* DNA polymorphisms are likely associated with genetic susceptibility to COVID-19.[95]

The gene *ApoE*, located on 19q13.32, is one of the highly co-expressed genes in type II alveolar cells in the lungs, which has been investigated in regard to COVID-19 prognosis.[96] Kuo et al. showed that the *ApoE e4* genotype predicted severe COVID-19 in the UK Biobank community cohort independently of the pre-existing CVD, dementia and type 2 diabetes.[97] Variations in human leukocyte antigen (HLA) have also been determined concerning the identification of individuals at higher risk of the disease.[98] HLA-B*46:01 had the fewest predicted binding peptides for SARS-CoV-2, suggesting that individuals with this allele could be particularly vulnerable to COVID-19. Conversely, HLA-B*15:03 showed the greatest capacity to present highly conserved SARS-CoV-2 peptides, which are shared among common human coronaviruses.[98] The 3p21.31 gene cluster has been identified as a genetic susceptibility locus in patients with COVID-19-dependent respiratory failure. The potential involvement of the ABO blood group system was confirmed in a study involving 1,980 patients with COVID-19 and a severe disease course, defined as respiratory failure.[87]

Recently, there has been much interest in interferon (IFN) research in regard to the SARS-CoV-2 infection. Available data implicates the importance of type I IFN (IFN-I) signaling, including defects in IFN-I gene expression in defense against the SARS-CoV-2 infection, and suggests that the inherited deleterious variants may explain the subset of severe COVID-19.[99] Zhang et al. and Bastard et al. revealed mutations in genes that belong to the Toll-like receptor 3 (TLR3) and IFN-I signaling pathways, leading to undetectable levels of interferon alpha (IFN-α) in blood plasma during coronavirus infection, thus linking the mutations to defective IFN-α production and COVID-19 severity.[99,100] It has been shown that the degree of an increase of uracil in SARS-CoV-2 variants correlates with the enhanced production of TNF-α and IL-6 when compared with stimulation with the single-stranded RNA (ssRNA) sequence of the virus isolated in Wuhan. Thus, RNA editing has been stated a factor for mutation bias in SARS-CoV-2 variants, which could affect the production of host inflammatory cytokines and influence SARS-CoV-2 overreactivity.[101]

The COVID-19 risk factors discussed in this paper are presented comprehensively in Table 1.

Conclusions

Coronaviruses, including SARS-CoV-2, are characterized by fewer gene mutations than other RNA viruses. Although differences in within-host diversity between mild and severe COVID-19 cases have been revealed, direct factors determining COVID-19 severity are not yet fully described. Therefore, the accurate evaluation of the role of SARS-CoV-2 variant-dependent and host-dependent risk factors for COVID-19 severity and fatality may help to identify potential drug target candidates for further study and bring hope for a breakthrough in COVID-19 treatment.

Table 1. Risk factors associated with coronavirus disease 2019 (COVID-19)

Risk factor types	Risk factors
Demographic and social	– older age – male gender – race (Black, South Asian, minority ethnic groups) – nursing home residents – in-center communities (dialysis)
Clinical	– obesity – comorbidities (CVD, HTA, DM, COPD, asthma, CKD/dialysis, neoplasms, sickle-cell disease – immunocompromised state from solid-organ transplant – dementia – smoking – pregnancy – initial SOFA score >2 – dyspnea, respiratory rate >24 breaths/min – high amount of viral load
Laboratory	– elevated classical markers (CRP, ferritin, IL-6, D-dimer, IL-2, IL-7, IL-10, G-CSF, TNF-α, creatinine, AST, ALT, blood urea nitrogen, troponin, creatine kinase, HbA_{1c}) – elevated new markers (IP-10, MCP-1, MIP1A) – leukocytosis – lymphopenia – high neutrophil-to-lymphocyte ratio
Human genetic	– ABO blood group – Rh positive blood type – ACE, TMPRSS2, DPP4, *ApoE e4* gene polymorphisms – HLA variations – defects in IFN-I gene expression
SARS-CoV-2 genetic	– within-host SARS-CoV-2 diversity – RNA editing – *ACE2* variants (including rs146598386, rs73195521, rs755766792, etc.) – ORF3a protein mutations

SARS-CoV-2 – severe acute respiratory syndrome coronavirus-2; CVD – cardiovascular diseases; HTA – arterial hypertension; DM – diabetes mellitus; COPD – chronic obstructive pulmonary disease; CKD – chronic kidney disease; SOFA – Sequential Organ Failure Assessment; CRP – C-reactive protein; IL – interleukin; G-CSF – granulocyte colony-stimulating factor; TNF-α – tumor necrosis factor alpha; AST – aspartate aminotransferase; ALT – alanine aminotransferase; HbA_{1c} – glycated hemoglobin; IP-10 – interferon gamma-inducible protein-10; MCP-1 – monocyte chemoattractant protein-1; MIP1A – macrophage inflammatory protein-1alpha; ACE – angiotensin-converting enzyme; TMPRSS2 – transmembrane protease serine 2; DPP4 – dipeptidyl peptidase-4; HLA – human leukocyte antigen; IFN-I – type I interferon; ACE2 – angiotensin-converting enzyme 2.

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Reviews

Oral manifestations of COVID-19: Brief review

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Abstract

The infection with a new type of virus – severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – called coronavirus disease 2019 (COVID-19) was first described in December 2019, in Wuhan, China. Due to the gastrointestinal mucosa tropism of the virus, an attempt was made to describe the oral manifestations of the SARS-CoV-2 infection. Angiotensin-converting enzyme 2 (ACE2), which permits the attachment of the virus, is present also in the oral cavity. There are many symptoms in the oral cavity; among them, the most prevalent ones are dysgeusia (taste disorders), oral pain, the exacerbation of autoimmune diseases as well as the herpes simplex virus (HSV) and varicella zoster virus (VZV) infections. Ulcerations and aphthous stomatitis are also often mentioned. The research shows that there are many oral symptoms in COVID-19, but the coexistence with the main disease has not been fully stated and understood. There is still no clearance on whether the oral symptoms are the manifestations of the disease or occur due to the loss of the immune response. Therefore, further studies on this subject should be conducted.

Key words: COVID-19, SARS-CoV-2, oral cavity, angiotensin-converting enzyme 2 receptor

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Introduction

The novel coronavirus pneumonia was first observed in December 2019 in Wuhan, in the Hubei Province of China. World Health Organization (WHO) named the virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease – coronavirus disease 2019 (COVID-19). The most common symptoms are fever, cough, shortness of breath or difficulty in breathing, fatigue, and muscle or body aches. Less common features, such as headache, the loss of taste or smell, a sore throat, diarrhea, and nausea or vomiting, may be present. The severity of symptoms depends on the time of exposure to the virus, the patient's age and gender as well as the coexisting diseases. The contact and droplet modes of transmission of the virus are well known. The gastrointestinal mucosa tropism of SARS-CoV-2 has been proven by biopsies. Due to the presence of the virus in stool, gastrointestinal transmission is also possible.[1–4]

The COVID-19 virus binds to 2 main host cell receptors – angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (also called transmembrane serine protease or TMPRSS2).[2] The severity of the coronavirus infection depends on many interactions, i.e., virus attachment, ACE2 receptor recognition, protease cleaving, and membrane fusion. The ACE2 receptor is highly expressed in the lung alveolar type 2 (AT2) cells, the esophagus, and the epithelial cells of the ileum and the colon. Apart from the above, ACE2 can also be found in the absorptive enterocytes from the ileum and the colon.[1]

In the oral cavity, those receptors are present mostly on the tongue and in the salivary glands.[3] They also occur in the nasopharynx, oral and nasal mucosa.[4] The course of infection is based on viral coagulopathy, which includes the consumption of platelets, thrombin and increased fibrin degradation products. The specific mechanism(s) of this phenomenon has not been fully understood. The treatment modalities have not been stated either.[2] It is also a well-known fact that patients with autoimmune diseases are predisposed to the SARS-CoV-2 infection.[5]

Nostril and throat swab are the most common diagnostic tools. The molecular polymerase chain reaction (PCR) tests enable finding the SARS-CoV-2 genes. In 42.9–81.8% of patients with a negative throat swab, the result might be positive when the probe is taken from the stool.[6]

The aim of this review was to briefly present the possible symptoms of COVID-19 in the oral cavity.

Material and methods

The PubMed database was searched to achieve the recent information, dating from December 2019 to October 2020. The key words were: 'COVID-19' and 'oral cavity manifestations'. Eighty-nine articles were found; among them, only 68 were full-text articles and referred to the studied topic. The chosen articles were in English only and were peer-reviewed. Twenty-one articles were written in a language other than English and were not taken into consideration. In the last step, the papers without clear background were excluded.

Oral manifestations

During the infection, SARS-CoV-2 is detected in saliva, so the salivary gland transmission of the virus is also possible.[1] The oral cavity, as part of the gastrointestinal duct, is also rich in ACE2 receptors, especially in the salivary glands and the dorsum of the tongue. Most of the symptoms present in the oral cavity are due to the impaired immune system and/or susceptible oral mucosa.[3]

The frequency of oral manifestations of COVID-19 is not known, but a huge study of 666 patients suggests that oral cavity findings are present in 25.65% of cases.[7,8] It is hypothesized that mild severity of disease is related to no symptoms or minor symptoms of the disease.[7,8] The common symptoms are blisters, ulcerations and desquamative gingivitis.[9] The ulcerations of the tongue occur quite often. The observations show that most of them are non-bleeding, but painful. They are always present on the dorsum or side of the tongue. Only in 15% of patients, tongue ulcerations develop on the ventral surface.[10] All the ulcerations and wounds may also result from the formation of thrombi and vasculitis in the oral cavity.[11] Doctors reported white plaque on the dorsum of the tongue during the SARS-CoV-2 infection as well.[2] The plaque did not react to antifungal treatment. It was retrospectively recognized as geographic tongue with an exacerbation during COVID-19. Additionally, multiple pinpoint yellowish ulcers on the dorsum of the tongue were present; those were probably the late-stage ulcers of the herpetic infection.[2] Herpes simplex virus (HSV) ulcerations are usually associated with stress due to presence of the disease and are not a specific symptom of the SARS-CoV-2 infection.[12] Frequently, ulcerations are combined with swelling.[13] Herpes zoster (HZ) disease might also be manifested.[10] Other oral symptoms might be explained through the aggravation of immune/defense mechanisms toward the present lesions. The most commonly observed are pemphigus, lichen planus, pemphigoid, and Sjögren's syndrome.[4] A specific set of symptoms, called Kawasaki-like symptoms, is the most severe oral manifestation. This includes erythema, dryness, the fissuring, peeling, cracking, and bleeding of mucosa and the lips, and strawberry tongue. Apart from oral manifestations, cervical lymphadenopathy is also observed.[14] In the case of COVID-19, the mimic of Kawasaki syndrome is called multisystem inflammatory syndrome in children (MIS-C) or hyperinflammatory shock. It is observed mainly in children. A sore throat, malaise, diarrhea and abdominal sensations, anorexia, rash skin, and conjunctivitis are other symptoms.[15]

Fungal infections are also one of the common oral manifestations of SARS-CoV-2, probably caused by lower immunity.[16] It has to be mentioned that a case of a neonate with COVID-19 and oral cavity candidiasis has been described.[17]

The most frequent locations of the COVID-19 symptoms are the palate and the tongue, followed by the gums and the lips. Patients report pain in 75% of cases.[18] Oral lesions heal within 3–21 days, either spontaneously or through topical treatment and oral hygiene.[19]

Most of the lesions in the oral cavity are prior to COVID-19 infection or result from the applied treatment.[3] Some patients may present with problems regarding soft tissues, saliva production and neurologically based oral sensations, even after a full recovery from COVID-19.[4] It is stated that olfactory and taste disturbances are the only oral symptoms of COVID-19. Dysgeusia and the loss of scent are caused by the edema of the respiratory epithelium.[12] Most of the patients (91%) report taste changes before other symptoms occur.[20,21] As many as 33.9% of patients present taste or olfactory disorders (e.g., anosmia)[20] and 18.6% – both of these.[21] Other researches show that 25% of patients reported impaired taste, 15% had burning sensations and 20% difficulty in swallowing.[22] Ageusia is observed in 24% of patients, hypogeusia in 35% and dysgeusia in 38%.[19] Taste disorders are more common in women than men.[19] It has also been reported that instead of the loss of taste, metallic taste was felt.[14]

A strong correlation between oral cavity manifestations and COVID-19 has also been shown. The appearance of oral lesions depends on the severity of the main disease. During the treatment of the viral infection, oral symptoms recede as well.[23]

All the symptoms mentioned above are collected in Table 1.

The recent research by Emodi-Perlman et al. shows that also the temporomandibular joint and muscles are involved in COVID-19.[24] The authors proved that the worldwide situation had increased the frequency of anxiety and resulted in the rise of temporomandibular disorders and bruxism.[24]

Table 1. Oral manifestations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection[2,3,7,8,13,14,19]

Region of the oral cavity	Symptoms
Tongue dorsum	white plaque and erythematous plaque, yellowish ulcerations (HSV), exacerbation of geographic tongue, candidiasis, thrush, ulcers, transient lingual papillitis, glossitis, lateral indentations, patch depapillation
Oral mucosa	ulcerations, petechiae, stomatitis (specifically aphthous stomatitis), exacerbation of autoimmune disorders, blisters, macules, mucositis
Gingivae	gingivitis, desquamative gingivitis
Other	dysgeusia (taste disorders), xerostomia, oral pain, erythematous surface in the oropharynx and on the hard palate, oral petechiae, a sore throat, Kawasaki-like disease, pruritus

HSV – herpes simplex virus.

Conclusions

It has neither been confirmed nor disproven that the oral cavity is a place of manifestation of the SARS-CoV-2 infection and COVID-19. We still have to remember that oral–fecal transmission is possible, especially that the virus is detected in stool months after the beginning of the infection, even after the respiratory symptoms disappear.[25,26] A conclusion that oral manifestations may not be symptomatic of SARS-CoV-2, but may result from the exacerbation of other diseases, should be made.[27] It also has to be remembered that the oral cavity is full of ACE2 receptors, which have a strong affinity to the COVID-19 virus.

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Reviews

Role of mental state in temporomandibular disorders: A review of the literature

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Abstract

Temporomandibular disorders (TMD) constitute a heterogeneous group of disorders concerning temporomandibular joints (TMJ) and the surrounding structures. The etiology is multifactorial, and comprises biological factors (e.g., internal derangements in TMJ), psychological factors (e.g., depression, anxiety and stress) and social factors (e.g., a learned response to pain). In accordance with the biopsychosocial model of health and illness, psychological factors are recognized as highly significant in the development of TMD.

The aim of this review was to present the role of chosen mental disorders (depression, anxiety) in TMD and their significance for dental practitioners in the light of current knowledge. The PubMed, Scopus and Web of Science databases were searched for relevant studies. Finally, 22 studies were included in this review. The gathered literature shows convincing evidence that mental derangements play a significant role in TMD by influencing the onset of the disorder, the course of the condition and the patient's response to treatment. However, the precise role of each mental disorder still requires further clarification.

Key words: depression, anxiety, temporomandibular disorders, mental disorders

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Introduction

'Temporomandibular disorders' (TMD) is an umbrella term for a heterogeneous group of disorders concerning temporomandibular joints (TMJ) and the surrounding structures. Temporomandibular disorders are the second most common cause of pain in the orofacial region after toothache.[1] The prevalence of TMD in the general population is estimated at around 40%.[2] In young adults, the prevalence of TMD symptoms varies from 42.9% up to 60%.[3–7] The most common symptoms are: pain of the masticatory muscle or in the joint area; headache; reduced mobility of the mandible, and joint sounds.[8] The etiology of TMD is multifactorial,[9–11] and comprises biological factors (e.g., internal derangements in TMJ), psychological factors (e.g., depression, anxiety and stress) and social factors (e.g., a learned response to pain).[12] The Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) introduced by Dworkin and LeResche in 1992,[13] later updated to Diagnostic Criteria for Temporomandibular Disorders (DC/TMD), is an internationally accepted tool for diagnosing TMD based on the biopsychosocial model of health and illness. The RDC/TMD and the latest DC/TMD function as a two-axis system. Axis I focuses on diagnosing biological factors based on physical examination, and Axis II refers to psychosocial factors, studied by means of a series of questionnaires.[13] The two-axis structure proposed in this diagnostic tool brings out the importance of psychosocial factors in TMD, which are still often underestimated or unknown by various clinicians working in the field of dentistry.

The aim of this review was to present the role of chosen mental disorders (depression, anxiety) in TMD and their significance for dental practitioners in the light of current knowledge.

Material and methods

The authors searched 3 electronic databases – PubMed, Scopus and Web of Science – for articles describing the relationship between anxiety or depression and TMD. Only original, English-language articles were included. Also the bibliography of the obtained studies was searched for relevant articles. Then, the retrieved papers underwent a qualitative analysis. Finally, 22 articles were included in this review.

Results and discussion

Mental disorders, such as depression or anxiety, are considered by some authors as the potential causes of TMD symptoms.[14,15] They play an important role in the onset of TMD and its perpetuation.[16–18] Some authors consider psychosocial comorbid conditions as predictors of a less favorable response to classical therapy.[19]

The precise mechanisms linking psyche and TMD are unknown. Potentially, stress may alter the threshold of pain perception in the central nervous system, increase the intensity of parafunctional habits as well as masticatory muscle fatigue and tightness, and initiate the disorder.[14,15] Or the other way round – pain (especially chronic), through the constant input of painful stimuli, induces central sensitization and causes permanent changes in the central nervous system.[20] Pain and psychological distress seem to create a dynamic vicious circle, in which mental disorders intensify the perceived pain and the perceived pain worsens the course of mental disorders. In many cases, it is hard to distinguish the cause from the effect.[21] The mechanism may also be associated with the dysregulation of the hypothalamic–pituitary–adrenal axis, leading to the production of excessive amounts of stress hormones, such as cortisol and catecholamines. The axis hormones are associated with mental disorders, such as depression, and other somatic illnesses, such as diabetes, hypertension or facial pain.[22–24] Other studies confirmed that patients diagnosed with TMD presented with statistically significantly higher levels of stress hormones in comparison with healthy controls.[25,26]

Depression

A depressive episode is characterized by subsequent symptoms: low mood; loss of interest or no sense of pleasure; psychomotor agitation or retardation; fatigue or loss of energy; significant changes in weight; strong belief in own worthlessness or a sense of guilt; impaired concentration; and hypersomnia or insomnia.[27–29] Patients suffering from depression are at higher risk of committing suicide.[30]

In the last 20 years, the prevalence of depression has increased by 49.86% and it is estimated that during lifetime, 20% of the society will develop this illness.[31–33]

The most commonly used questionnaires diagnosing depression are: the Hospital Anxiety and Depression Scale (HADS)[1]; the Patient Health Questionnaire-9 (PHQ-9)[34]; the Symptom Checklist-90-Revised (SCL-90R)[35,36]; and the Beck Depression Inventory (BDI).[37] The usage of those questionnaires as diagnostic tools is very convenient and time-saving from the point of view of the doctor. Still, it has to be marked that questionnaires can only serve as screening tools for certain psychological disorders, and indicate patients that need a definitive diagnosis and treatment provided by the psychiatrist.[1,38]

Patients with TMD present with higher levels of depression as compared to healthy controls.[29,39,40] Some studies divided TMD patients based on pain, reporting significantly higher levels of depression in patients with painful TMD than in the case of non-painful TMD.[36,41,42] What is worth noticing in those studies is the fact that the precise localization of pain (diagnosis from Axis I) was not significant.[36,41,42] Others divided patients based on the duration of the symptoms, reporting significantly higher levels of depression in patients with chronic TMD than those with acute TMD.[20,38] In the above-mentioned studies, chronic TMD was defined as lasting at least 3 months. One of the studies focused on changes in the perceived symptoms of TMD at the time of higher and lower levels of depression and anxiety.[43] A significant difference was found only in the maximum mouth opening without pain and the level of self-estimated mandibular impairment. The maximum mouth opening without pain was decreased and the level of self-estimated mandibular impairment was increased at the time of a high level of depression. On the other hand, non-significant differences were found in the number of tender points or the electromyographic activity of the masticatory muscle, which seemed not to depend on the level of anxiety and depression.[43]

Korszun et al. conducted an interesting study, in which patients with chronic facial pain underwent full medical and psychiatric assessment according to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria.[26] The percentage of patients diagnosed with depression or symptoms characteristic of depression was statistically significantly higher among patients with chronic facial pain than in healthy controls. Interestingly, fatigue and insomnia were the most common symptoms, reported by 92% of patients with a diagnosis of depression and 50% of patients presenting with only some depressive symptoms. By comparison, 38% of controls reported those symptoms. An important issue is that 50% of patients fulfilling the DSM-IV criteria for depression received this diagnosis for the first time, despite various previous consultations. This fact might indicate that the number of patients with TMD requiring a specialist psychiatric consultation is underestimated by various practitioners. No statistically significant differences in the occurrence of depression between TMD patients and chronic facial pain patients of different origin was found, either. Thus, the significant variable correlated with depression could be pain, not TMD.[26,44] Several studies suggest that depression and chronic pain syndromes share the same neurological pathways and neurotransmitters (such as norepinephrine, glutamate and serotonin), and involve the same brain structures (such as the anterior cingulate cortex, the prefrontal cortex, the amygdala, and the hippocampus).[45–47] Furthermore, both chronic pain and depression aggravate each other, and cause maladaptive changes in the brain function and structure.[46,47] Chronic pain intensifies depressive symptoms by causing distress.[46] Depression on the other hand may be a result of monoamine neurotransmitter deficiency, which leads to increased sensitivity to painful stimuli.[45] It is also suggested that depression may be considered as an inflammatory process in the brain due to increased levels of pro-inflammatory cytokines, which results in higher pain sensitivity.[46] Therefore, early diagnosis and proper treatment are crucial.

Another interesting finding is that young children who had contact with adults with depression are at increased risk of the development of painful TMD in their early adulthood.[48]

Table 1 presents a summary of data regarding depression from the most important studies included in the review.

Anxiety

Anxiety is characterized by a sense of worry, difficult to control and causing a feeling of restlessness, fatigue, a sense of tension, nervousness, and sleep disturbance.[27,49] In some studies, in which the Spielberger State-Trait Anxiety Inventory (STAI) was used, 2 types of anxiety were distinguished: state-anxiety, which is associated with the current level of anxiety and is transitory; and trait-anxiety, which expresses one's personality and is more stable during lifetime.[50] Anxiety is recognized as the most common mental disorder in the European Union and it is 3 times more frequently diagnosed in women than men.[51]

Studies employ various questionnaires to diagnose anxiety. These are: HADS[1,37]; STAI[15,37,52,53]; the Beck Anxiety Inventory (BAI)[37,52]; the Generalized Anxiety Disorder-7 (GAD-7)[34]; and SCL-90R.[35,36]

The role of anxiety in TMD is still rather controversial. Many studies suggest the existence of a correlation between TMD and anxiety,[3,37,54–56] but other researchers present contradictory results,[1,20,57–59] seeing anxiety as a less important factor in the case of TMD patients than depression.[1,20] The different results of the presented studies may be explained by differences in many important variables, such as the population under study, the study design and the questionnaire used.

Those authors who managed to find a correlation between TMD and anxiety reported significantly higher levels of anxiety in patients with TMD as compared to healthy controls. The individuals affected by anxiety were up to 5 times more prone to develop TMD than non-TMD patients.[48] A correlation was found in both acute[60] and chronic TMD.[61] Severe anxiety levels increased by twice the probability of chronic pain, disability and depression,[53] and the level of anxiety correlated with the duration of the disorder.[20]

Regarding one of the subtypes of anxiety, the analyzed articles were consistent. Trait-anxiety occurred more often in patients with TMD than healthy controls.[15,53]

Table 1. Summary of data regarding depression from the most important studies included in the review

Study	Population	Study group	Control group	TMD diagnosing tool	Psychiatric diagnosing tool	Correlation	Conclusion
Giannakopoulos et al. (2010)[1]	*N* = 222 61 men 161 women	chronic, painful TMD	non-painful TMD, patients without chronic facial pain	RDC/TMD	HADS	+	significantly higher levels of depression in the study group
Cao et al. (2020)[20]	*N* = 830	TMD subgroups	TMD subgroups	RDC/TMD	DASS-21	+/–	significantly higher levels of depression only in the chronic, painful TMD subgroup
Korszun et al. (1996)[26]	*N* = 72	TMD	chronic facial pain	University of Washington criteria	psychiatric examination	–	no significant correlation found
Simoen et al. (2020)[34]	*N* = 243 52 men 191 women	painful TMD	non-TMD	DC/TMD	PSQ-9	+	significantly higher levels of depression in the study group, unrelated to gender
De la Torre Canales et al. (2020)[36]	*N* = 737 150 men 587 women	painful TMD	non-painful TMD	RDC/TMD	SCL-90R	+	significantly higher levels of depression in the study group
Reiter et al. (2015)[38]	*N* = 207	chronic TMD	acute TMD	RDC/TMD	SCL-90R	+	significantly higher levels of depression in the study group, additionally, more severe pain and disability
Fernandes et al. (2013)[41]	*N* = 224	painful TMD	non-painful TMD or non-TMD	RDC/TMD	SCL-90	+	significantly higher levels of severe depression in the study group
Maślak-Bereś et al. (2019)[42]	*N* = 260	TMD subgroups	non-TMD	RDC/TMD	BDI	+	significantly higher levels of depression in patients with painful TMD, no differences between the study groups
Calixtre et al. (2014)[43]	*N* = 116 32 men 84 women	TMD	TMD	RDC/TMD	HADS	+	significant differences found only in the maximum mouth opening without pain and the level of self-estimated mandibular impairment at the time of higher levels of depression
Manfredini et al. (2009)[44]	*N* = 96 21 men 75 women	TMD subgroups	TMD subgroups	RDC/TMD	SCL-90R	–	no significant correlation found

TMD – temporomandibular disorders; RDC/TMD – Research Diagnostic Criteria for Temporomandibular Disorders; DC/TMD – Diagnostic Criteria for Temporomandibular Disorders; HADS – Hospital Anxiety and Depression Scale; DASS-21 – Depression, Anxiety and Stress Scale-21; PHQ-9 – Patient Health Questionnaire-9; SCL-90R – Symptom Checklist-90-Revised; SCL-90 – Symptom Checklist-90; BDI – Beck Depression Inventory.

The odds of TMD correlated with the level of trait-anxiety, regardless of age, gender or the level of education. Patients suffering from trait-anxiety at a moderate level were at higher risk of TMD, while severe trait-anxiety doubled the risk.[53]

In one of the analyzed articles, higher state-anxiety levels correlated with an increased risk of painful TMD[53] whereas in another study, they did not.[15] This inconsistency can be easily explained, because, as mentioned before, state-anxiety concerns the momentarily perceived anxiety, which can be influenced by other psychosocial factors, not taken into consideration in either of the presented articles.

Similarly to depression, anxiety does not show any correlation with Axis I diagnosis, leading to the conclusion that the localization of the symptoms is insignificant.[35,50,53]

There is weak evidence that the level of anxiety tends to decrease significantly after 1 month of treatment, regardless of the type of therapy used (occlusal splint therapy, manual therapy, counseling, or the combination of occlusal splint therapy and counseling).[52]

Table 2 presents a summary of data regarding anxiety from the most important studies included in the review.

Limitations

The usage of questionnaires as the only diagnostic tools for mental disorders is an important limitation of the included studies. The lack of further psychiatric confirmation may lead to misdiagnosis, biasing the presented results. Furthermore, questionnaires screen only for certain symptoms and do not provide a precise psychiatric diagnosis.

Conclusions

Evidence supporting a correlation between mental disorders, such as depression and anxiety, and TMD is convincing and numerous. The derangements

Table 2. Summary of data regarding anxiety from the most important studies included in the review

Study	Population	Study group	Control group	TMD diagnosing tool	Psychiatric diagnosing tool	Correlation	Conclusion
Giannakopoulos et al. (2010)[1]	*N* = 222 61 men 161 women	chronic, painful TMD	non-painful TMD, patients without chronic facial pain	RDC/TMD	HADS	+	no significant correlation found
Casanova-Rosado et al. (2006)[3]	*N* = 506	TMD	non-TMD	RDC/TMD	LS	+	significantly higher levels of anxiety in the study group
Monteiro et al. (2011)[15]	*N* = 150 117 men 33 women	TMD (chronic orofacial pain)	non-TMD	RDC/TMD	STAI	+	significantly higher levels of trait-anxiety in the study group
Cao et al. (2020)[20]	*N* = 830	TMD subgroups	TMD subgroups	RDC/TMD	DASS-21	+/–	significantly higher levels of anxiety only in the chronic, painful TMD subgroup
Simoen et al. (2020)[34]	*N* = 243 52 men 191 women	painful TMD	non-TMD	DC/TMD	GAD-7	+	significantly higher levels of anxiety in the study group, unrelated to gender
De la Torre Canales et al. (2020)[36]	*N* = 737 150 men 587 women	painful TMD	non-painful TMD	RDC/TMD	SCL-90R	+	no significant correlation found
Machado de Resende et al. (2020)[37]	*N* = 120 48 men 72 women	TMD	non-TMD	RDC/TMD	BAI, STAI, HADS	+	significantly higher levels of anxiety in the study group
Reiter et al. (2015)[38]	*N* = 207	chronic TMD	acute TMD	RDC/TMD	SCL-90	–	no significant correlation found
Reissmann et al. (2014)[53]	*N* = 1,208 436 men 772 women	TMD	non-TMD	RDC/TMD	STAI	+	significantly higher levels of anxiety in the study group

LS – Likert Scale; STAI – State-Trait Anxiety Inventory; GAD-7 – Generalized Anxiety Disorder-7; BAI – Beck Anxiety Inventory.

influence the onset of TMD, the course of the disease and a response to treatment. However, the precise role of each mental disorder still requires further clarification.

Screening for mental disorders in TMD patients by means of questionnaires in general dentists' offices is highly recommended.

Depression correlates with TMD; the presence or absence of orofacial pain might by a more important factor than a diagnosis of TMD.

A correlation between anxiety and TMD is rather controversial and depends on various factors, such as the study design, the population under study, the control group, etc. Future studies should focus on investigating the circumstances in which the correlation is significant.

In further research, precise causal relationships should be established between depression, anxiety and TMD, along with defining the prevalence and coexistence of the above-mentioned conditions. A diagnosis of mental disorders should be confirmed by psychiatric examination, not only based on questionnaires.

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