Dental and Medical Problems

BIMONTHLY ISSN 1644-387X (PRINT) ISSN 2300-9020 (ONLINE) www.dmp.umw.edu.pl

2024, Vol. 61, No. 5 (September–October)

Impact Factor (IF) – 2.7
Ministry of Science and Higher Education – 70 pts

Wroclaw Medical University

Dental and Medical Problems

ISSN 1644-387X (PRINT) ISSN 2300-9020 (ONLINE) www.dmp.umw.edu.pl

BIMONTHLY
2024, Vol. 61, No. 5
(September–October)

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

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

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

Manuscript editing
Joanna Gudarowska
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Dental and Medical Problems has received financial support from the resources of the Ministry of Science and Higher Education within the "Social Responsibility of Science – Support for Academic Publishing" project based on agreement No. RCN/SP/0493/2021.

Ministerstwo
Edukacji i Nauki

Indexed in: PubMed/MEDLINE, Web of Science, Clarivate Journal Citation Report, Scopus, ICI Journals Master List, DOAJ, WorldCat, Embase, Polska Bibliografia Naukowa, EBSCO, Crossref, CLOCKSS

Typographic design: Monika Kolęda, Piotr Gil
Cover: Monika Kolęda
DTP: Adam Barg
Printing and binding: Drukarnia I-BiS Bierońscy Sp.k.

Dental
and Medical Problems

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ISSN 1644-387X (PRINT)
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Revisiting peri-implant diseases in order to rethink the future of compromised dental implants: Considerations, perspectives, treatment, and prognosis

Gustavo Vicentis de Oliveira Fernandes[1,A–F], Bruno Gomes dos Santos Martins[2,A–F], Javier Flores Fraile[2,A–F]

[1] Department of Periodontics, Missouri School of Dentistry and Oral Health, A.T. Still University, St. Louis, USA
[2] Surgery and Odontostomatology, University of Salamanca, Spain

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):637–640

Address for correspondence
Gustavo Vicentis de Oliveira Fernandes
E-mail: gustfernandes@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on April 9, 2024
Reviewed on April 11, 2024
Accepted on April 15, 2024

Published online on October 24, 2024

Cite as
Fernandes GVdO, Martins BGdS, Fraile JF. Revisiting peri-implant diseases in order to rethink the future of compromised dental implants: Considerations, perspectives, treatment, and prognosis. *Dent Med Probl.* 2024;61(5):637–640. doi:10.17219/dmp/187215

DOI
10.17219/dmp/187215

Keywords: therapeutics, prognosis, dental implants, peri-implantitis, bone–implant interface

Once present, peri-implantitis is difficult to fully eliminate. Surgical interventions show some promising results in fighting the disease, but for the time being, prevention remains the strongest tool.

Introduction

Peri-implant disease (PID) is a global term for biological responses to local aggression on tissues around dental implants. Similar to gingivitis and periodontitis, peri-implant mucositis (PIM) and peri-implantitis (PI) were first coined in 1993 at the 1st European Workshop on Periodontology in a consensus report.[1] Peri-implant disease encapsulates all inflammatory and immune system-mediated responses around the tissues of the osseointegrated implant. Peri-implant mucositis refers to the reversible inflammation of these tissues. Peri-implantitis presents the same inflammatory status involving soft and hard tissues, which may progress to severe bone loss in its advanced phases.[1,2] At the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions,[2] the definition of PI was updated to a plaque (polymicrobial)-associated disease that occurs around the osseointegrated implant.[1–4]

Peri-implant health, peri-implant mucositis and peri-implantitis

The expected clinical scenario in a healthy peri-implant location is an erythema-free area with no bleeding on probing (BoP) and no edema or suppuration. The main assessment tools are visual/clinical observation and palpation. If erythema, edema or pus are present, a periodontal/peri-implant probe should be used to assess the probing depth (PD).[2]

Peri-implant mucositis is clinically characterized by erythema, edema and/or suppuration. After gentle probing, bleeding is often observed on the implant sulcus. An increased PD is mostly due to tissue swelling, as mucositis does not present with bone loss. Plaque/biofilm is the main etiological factor for PIM, as observed in animal and human experimental studies.[2] Even though there are some human studies evidencing that mucositis can resolve after treatment, some systematic reviews claim that total disease resolution is not predictable, and PIM progression to PI tends to occur.[5–7]

Peri-implantitis is believed to be the progression of PIM, showing the same clinical characteristics, but greater PD (>6 mm) and radiographic bone loss (RBL). Bone loss is usually directly related to the progression and severity of the disease. Poor biofilm control by the patient and the lack of compliance with follow-up recommendations have been shown to be risk-increasing behaviors.[6–10] Some other factors may play a role in the development of PI, such as smoking habits and diabetes[2]; occlusal overload, prosthodontic rehabilitation characteristics, the presence or absence of keratinized mucosa, titanium (Ti) particles, abutment micromovements, biocorrosion, and cement remnants seem to play some role in the progression of the disease, but high-quality studies fail to accurately determine the effects of these factors on PI progression.[2,5,11–13]

Prevalence and pathogenesis

Since dental implants are reported to have high survival rates (90–95% at >5 years),[14–17] it is only natural that we are observing an upward trend in their usage for the replacement of missing teeth. With regard to the above, it is logical to assume that the rate of complications will follow a similar trend. It has been clinically confirmed, with an increase in PI cases over the last 25 years.[1,2,5] Recent studies estimate a prevalence of approx. 20% for PI, which can rise up to around 47% if the incidence of PIM is considered.[17,18]

Over the last decade, some new theories have arisen about the true pathogenesis of PID. The main theory assumes that a foreign body reaction (FBR) always occurs around the osseointegrated implant, causing chronic low-grade inflammation. With the aggravation of this latent inflammation, peri-implant bone loss will be the ultimate result.[19] The main difference between the current, widely accepted theory and the FBR theory is the relevance of dental plaque accumulation and bacterial implication in the pathogenesis of the disease. The determination of one or another factor could dictate a paradigm shift in the treatment options for PI. There is still a lack of scientific evidence for FBR to be a unidirectional cause of PI, and it seems that plaque accumulation should not be underestimated.[20]

Treatment lines for peri-implant disease

According to the current knowledge about PI, the progression of the disease is plaque-dependent. Thus, treatment should focus on controlling and eliminating plaque.[1,5] With that being said, PI therapy comprises some initial steps, i.e., infection control and non-surgical interventions by the removal of plaque, through sub- and supragingival debridement, and finally follow-up evaluation. This treatment approach has been proven to be insufficient for treating true PI lesions, although it is successfully applied in the case of PIM.[2,5]

For true PI lesions, another stage is required, namely the surgical therapy phase. Surgical treatment encompasses flap elevation, implant surface detoxification/decontamination, and pocket/granulation tissue elimination if required. If it is justified by the type of the existing bone defect, a regenerative approach with the use of the available biomaterials and a membrane for guided bone regeneration (GBR) can also be applied.[5,11]

Several adjunctive therapies have been thoroughly studied, such as laser mono/combined application, the usage of antibiotics (locally or systemically), and alternative decontamination methods (implantoplasty, air polishing, the use of an ultrasonic apparatus and Ti brushes, and electrolytic decontamination with GalvoSurge®).[5]

It has been widely recognized that PI disease prevention is the best available choice, since treatment options are still not totally predictable and reliable. The best actions for preventing the development of PID, as per the latest guidelines,[11] are: (1) the proper evaluation of soft and hard tissues that will receive the future dental implant (the presence or absence of keratinized mucosa, the width of the available mucosa, the presence or absence of any gingival/bony defect, and bone availability for implant placement); (2) proper three-dimensional (3D) implant placement; (3) the proper planning and execution of the prosthodontic piece (allowing adequate cleanability for the patient); and (4) the proper establishment of a follow-up schedule for each patient, taking into consideration particular risk factors.

Adherence to oral health instructions and periodic follow-up appointments for supportive peri-implant care are believed to be key factors in maintaining peri-implant health and preventing the development of any PID.[1,2,11]

Treatment outcomes for peri-implant disease

An umbrella review published in 2022 by Martins et al. evaluated 9 systematic review articles encompassing 59 unique randomized controlled trials (RCTs).[5] The study

found that in treating PI lesions, non-surgical approaches had limited effects and could not stop the evolution of PI. Some clinical parameters might be improved, i.e., BoP and, to a lesser degree, PD. Non-surgical options were mostly recommended for treating PI within the first-stage intervention or treating PIM more efficiently. The greater the PD, the more limited the effects of non-surgical interventions appear to be. Combining non-surgical therapies with adjunctive methods (i.e., lasers and local antibiotic/antiseptic therapy) offered better clinical results, although some methods were controversial. Abrasive polishing with glycine powder, erbium-doped yttrium aluminum garnet (Er:YAG) laser application, debridement with an ultrasonic apparatus or curettes, and local antibiotics/antiseptics worked better when used in conjunction. Yet, none of them reduced the bacterial load at the implant surface enough to avoid the development of PI.[5]

Surgical techniques seem to be the best option to treat PI and hinder the development of the disease.[1,2,5,11] Resective interventions may improve clinical parameters and, to some extent, diminish the effects of inflammation (lower BoP and sulcus/pocket PD). Normally, resective techniques by themselves result in some kind of soft tissue/peri-implant tissue loss. Thus, a regenerative procedure may be recommended.[5]

Regenerative surgical techniques yield generally positive results, showing better clinical and radiographic outcomes in most high-quality studies.[21] Predicting the magnitude of improvement with any surgical technique is still difficult and disease recurrence is not uncommon. Patient-related outcome measurements are also rarely reported.[5,11] In the available literature, no specific material (i.e., membranes, bone substitutes or bioactive agents) is superior to another. No clear advantage with regard to clinical outcomes was found when comparing resective only and regenerative procedures.[5,11] Implantoplasty was the most effective implant surface decontamination method, but other concerns, such as Ti particles scattering during the procedure, still need to be investigated. The most recent European Federation of Periodontology (EFP) guidelines[11] as well as another study[1] corroborate the aforementioned findings. Table 1 summarizes the prevention, treatment and prognostic issues regarding PID.

Conclusions and considerations

As modern-day oral rehabilitation protocols for partially and fully edentulous patients rely more and more on implantology, preventing biological complications is a key factor for the success and longevity of implants. Preventing the development of PID seems to be the best path to avoid having to deal with the most serious version of the disease, PI. With the current knowledge, several steps can be taken toward the prevention of PID: (1) preoperative evaluation, especially examining the gingival and bone characteristics of the area; (2) the proper planning of implant placement in the correct 3D position (reverse

Table 1. Peri-implant disease (PID) – prevention, treatment, follow-up, and prognosis

PID	Prevention	Treatment	Follow-up	Prognosis
PIM/PI	(1) proper evaluation of soft and hard tissues that will receive the future dental implant (the presence or absence of keratinized mucosa, the width of the available mucosa, the presence or absence of any gingival/bony defect, and bone availability for implant placement); (2) proper 3D implant placement; (3) proper planning and execution of the prosthodontic piece (allowing adequate cleanability for the patient); (4) proper establishment of a follow-up schedule for each patient, considering all risk factors	–	–	extremely favorable
PIM	–	non-surgical procedures: abrasive polishing with glycine powder, Er:YAG laser application, debridement with an ultrasonic apparatus or curettes, and local antibiotics/antiseptics	moderate/severe PIM: at 3 months mild PIM: at 6 months	favorable
PI	–	non-surgical procedures: first-stage intervention non-surgical and surgical procedures (combination): abrasive polishing with glycine powder, Er:YAG laser application, debridement with an ultrasonic apparatus or curettes, and local antibiotics/antiseptics implantoplasty, Ti brushes and electrolytic decontamination with GalvoSurge®, and GBR	moderate/severe PI: at 6 weeks–3 months mild PI: at 3–6 months	poor/favorable

PIM – peri-implant mucositis; PI – peri-implantitis; 3D – three-dimensional; Er:YAG laser – erbium-doped yttrium aluminum garnet laser;Ti – titanium; GBR – guided bone regeneration.

planning); (3) correct prosthodontic planning, allowing the cleanability of the rehabilitated area; and (4) evaluating the patient comprehensively, taking into consideration their hygiene habits, as well as systemic factors, to correctly define a supportive care schedule for the maintenance of peri-implant health.

The available data on the actual treatment of PI are reliable and already provide rough guidelines for dealing with possible complications. Yet, due to the scarcity of high-quality evidence in the literature, knowledge on PI treatment still has to be expanded. In the last few years, we have witnessed great advancement with regard to understanding the physiopathology and progression pattern of the disease, but fully eliminating it still seems like a distant aspiration. Patient-related outcome measurements are also lacking in most of the available studies. Some promising decontamination methods are starting to be investigated, as well as extra surface treatment to reduce plaque accumulation on implants and abutments. The role of the potential release of Ti particles is also a point of interest, and further studies are required.

To conclude, once present, peri-implantitis is difficult to fully eliminate. Surgical interventions show some promising results in fighting the disease, but for the time being, prevention remains the strongest tool.

Take-home message

(1) Prevention is the key to the maintenance of peri-implant health. We suggest keeping the following schedule of periodontal follow-ups: 6 months for healthy individuals; 3–6 months for PIM cases (depending on the level of mucositis); and 3 months for PI cases.

(2) In cases of PI, full elimination still seems impossible; thus, depending on the implant length being compromised, prolonging the life of the implant is extremely questionable, which can result in implant removal.

(3) Surgical interventions for PI present better results than non-surgical activities; therefore, prognosis for the implant (depending on the level of involvement) may be unpredictable.

ORCID iDs

Gustavo Vicentis de Oliveira Fernandes
https://orcid.org/0000-0003-3022-4390
Bruno Gomes dos Santos Martins
https://orcid.org/0009-0003-8729-6616
Javier Flores Fraile https://orcid.org/0000-0003-1338-0551

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Original paper

Comparative evaluation of three different behavior management techniques among children aged 6–12 years in dental practice: A single-center, double-blind, randomized controlled trial

Priyanka Balakrishnan[A–F], Daya Srinivasan[A–F], Senthil Eagappan[A–F]

Department of Pediatric and Preventive Dentistry, Chettinad Dental College & Research Institute, Chennai, India

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):641–650

Address for correspondence
Priyanka Balakrishnan
E-mail: priyabalu1907@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on May 14, 2023
Reviewed on July 17, 2023
Accepted on July 24, 2023

Published online on October 3, 2024

Abstract

Background. Dental anxiety is characterized by distress in anticipation of dental visits, which may result in a child's refusal to undergo treatment and, ultimately, lead to parents' reluctance towards dental care.

Objectives. The aim of this study was to evaluate and compare the effectiveness of the tell-show-do technique, modeling technique and yogic relaxation technique in the reduction of dental anxiety among children aged 6–12 years.

Material and methods. The study was an interventional, parallel-group, single-center, double-blind, randomized controlled trial conducted on 120 children who required restorative treatment without the use of local anesthesia. The participants were selected based on specific inclusion and exclusion criteria and were randomly divided into 3 groups: group 1 – tell-show-do technique; group 2 – modeling technique; group 3 – yogic relaxation technique. The dental anxiety levels were evaluated 4 times using both physiological (oxygen saturation and pulse rate) and behavioral parameters (facial image scale (FIS) and the Face, Legs, Activity, Cry, and Consolability scale (FLACC)). The data was assessed by 2 blinded and calibrated specialists.

Results. Statistically significant differences were observed in all 4 parameters among the 3 groups. During both the intraoperative and postoperative periods, the oxygen saturation levels were significantly higher in the yogic relaxation technique group. Moreover, the yogic relaxation technique group exhibited lower pulse rates, FIS and FLACC scores compared to the tell-show-do and modeling technique groups.

Conclusions. The practice of yoga has a positive influence on the general health of the individual. Consequently, it can be considered one of the alternative behavioral modification techniques for the reduction of dental anxiety in children.

Keywords: anxiety, dental anxiety, behavior, yoga, dental fear

Cite as
Balakrishnan P, Srinivasan D, Eagappan S. Comparative evaluation of three different behavior management techniques among children aged 6–12 years in dental practice: A single-center, double-blind, randomized controlled trial. *Dent Med Probl.* 2024;61(5):641–650. doi:10.17219/dmp/169966

DOI
10.17219/dmp/169966

Introduction

Anxiety is characterized by disruptions in mood, thinking, behavior, and physiological processes, and is often misunderstood as fear. A planned procedure may be avoided by patients who are experiencing anxiety, due to its unpleasant nature.[1,2] Thus, anxiety is an emotional state that occurs before the actual encounter with the threatening stimuli, which is not always identifiable. On the other hand, fear is a response to a real or hypothetical threat or danger.[3] Dental anxiety is the 5th most common cause of anxiety.[4] The prevalence of dental anxiety is estimated to range from 6% to 20% in children between the ages of 4 and 18.[5] According to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV and the International Statistical Classification of Diseases and Related Health Problems,[3] a condition associated with fear of dentistry along with hypertension, apprehension, trepidation, and uneasiness is referred to as odontophobia.[3]

Dental anxiety is defined as "the distressed expectation of a visit to a dentist to the extent where a child might avoid treatment."[6] Early onset of dental anxiety is associated with a greater incidence of dental caries, which may ultimately lead to the early removal of the affected tooth due to the child's fear of attending the dental clinic. Compromised oral hygiene may result in a poor oral health-related quality of life. The sequence of events that an individual experiences during childhood will continue into adulthood through adolescence, having a negative impact on the oral health of the individual.[7–9]

Parent involvement has a significant impact on how well a child handles the pressures and stimuli associated with dental care.[10] The cognitive behavioral treatment, which is widely utilized in psychology and psychiatry for both adult and pediatric patients, serves as the foundation for the behavioral management approach.[11]

The provision of dental treatment for anxious children requires a cautious approach. In addition to meeting their healthcare needs, it should address any behavioral issues. Behavior management is an important aspect in the treatment of pediatric patients, as it inculcates the basic coping mechanisms and helps the child handle the stressful situation in a more acceptable way. It is not possible to achieve this coping mechanism in subsequent dental visits through the use of pharmacological methods. Thus, behavior modification through a non-pharmacological approach is the optimal choice for establishing long-term coping mechanisms in children.[12] However, if non-pharmacological methods of the behavior modification prove ineffective, pharmacological techniques such as sedation and general anesthesia can be employed.[13] Furthermore, yoga and meditation address a child's entire being, including their physical, mental, emotional, psychic, and spiritual well-being. Yoga provides total body synchronization and aids in the reduction of stress and anxiety. It is a simple and effective non-pharmacological intervention for the reduction of dental anxiety.[14]

Thus, the aim of this study was to compare 3 different behavior management techniques (the tell-show-do technique, modeling technique and yogic relaxation technique) for the reduction of dental anxiety in children aged 6–12 years.

Hypotheses

The alternative hypothesis suggests that the yogic relaxation technique has a better anxiolytic effect compared to the tell-show-do and modeling techniques.

The null hypothesis posits that the yogic relaxation technique has no or little anxiolytic effect in comparison to the tell-show-do and modeling techniques in reducing dental anxiety.

Material and methods

Study design

This study was designed as an interventional, parallel-group, single-center, double-blind, randomized controlled trial with a comparative analysis of the 3 groups among the pediatric population who presented to the Outpatient Department of Pedodontics and Preventive Dentistry (Chettinad Dental College and Research Institute, Chennai, India) between September 2022 and March 2023.

The study was approved by the ethical clearance board of Chettinad Academy of Research and Education, Chennai, India (approval No. IHEC-I/1302/22).

Study sample

The study sample included children aged 6–12 years who were in the pre-operational and concrete operational stages of cognitive development. A total sample size of 120 was calculated using G*Power software (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower), with an alpha error of 0.05, a beta power of 0.9 and an effect size of 0.33. The children and their parents reported with the chief complaint of dental caries. After initial clinical diagnosis and screening, children experiencing pain that required more invasive procedures were excluded from the study.

Inclusion criteria

The inclusion criteria for the study were as follows: children aged between 6 and 12 years; children attending their first dental visit; children requiring restorative treatment for their decayed teeth; and children with a Frankl behavior rating score of 2 or 3 (negative/positive).

Exclusion criteria

The study excluded children with mental or cognitive issues that could impair their comprehension or participation in the study, medically compromised children with systemic abnormalities that could influence the child's pulse rate and heart, children with a previous history of trauma to the teeth with pulpal involvement, children whose parents were not willing to consent to the treatment, and children requiring endodontic treatment or extraction for their chief complaint.

Study sample selection

After the comprehensive screening process, 64 boys and 56 girls (N = 120) were selected for inclusion in the study. The children were randomly allocated into 3 equal groups using the lottery method, based on the type of behavior management technique they received: group 1: tell-show-do technique; group 2: modeling technique; group 3: yogic relaxation technique.

Data collection

The following parameters were evaluated in the course of the study: oxygen saturation (P1); pulse rate (P2); facial image scale (FIS) (P3); and the Face, Leg, Activity, Cry, and Consolability scale (FLACC) (P4).

The parameters were measured at 4 different time points during the course of the dental procedure:

1. Preoperative period: before the dental procedure in the waiting room (T1);
2. After conditioning: after the application of the alloted behavior management technique in respective groups (T2);
3. Intraoperative period: during the dental procedure (T3);
4. Postoperative period: after the dental procedure in the waiting room (T4).

The Consolidated Standards of Reporting Trials (CONSORT) flowchart, which provides a visual representation of the study procedure, is presented in Fig. 1.

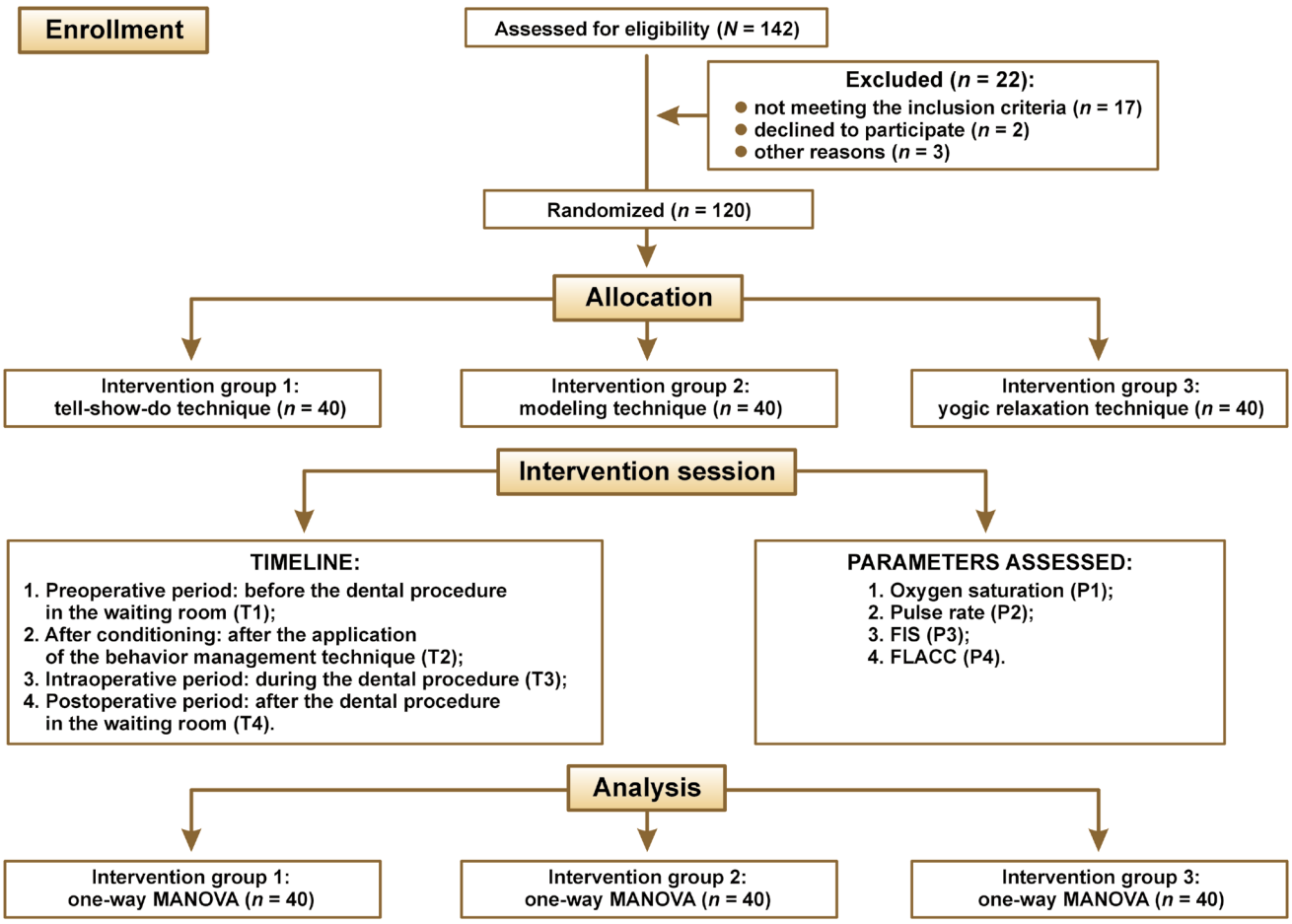

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flowchart of the participants' progress through the trial

FIS – facial image scale; FLACC – Face, Legs, Activity, Cry, and Consolability scale; MANOVA – multivariate analysis of variance.

Intervention

Group 1: Tell-show-do technique

For children in group 1, the nature of the procedure was explained in a non-threatening and intelligible manner, in the language that the children were most comfortable with. Each child was presented with the required armamentarium and the procedure was mimicked in a typodont. The child observed the procedure being carried out by the dentist (Fig. 2). After the observation phase, the child was reassured, and, with their consent, the dental procedure was initiated.

Group 2: Modeling technique

The child observed the procedure that was carried out on a model, which allowed the patient to gain insight into the nature of the procedure (Fig. 3). The model was either the child's sibling or another patient in the same age group who was undergoing similar treatment. Once the procedure had been completed on the model, the child was reassured and, with their consent, the dental procedure was initiated.

Group 3: Yogic relaxation technique

The child was instructed to perform 3 different yoga poses while seated in the dental chair:

1. Pancha Kosha (Fig. 4A);
2. Bhramari Pranayama (Fig. 4B);
3. Nadi Shodhana (Fig. 4C).

The child was asked to watch a virtually recorded visual demonstration of the yoga poses. Each pose was performed for 5 min under the supervision of the dental professional. Once the yoga session was completed, a 10-min interval was given, after which the dental procedure was initiated.

Fig. 2. Tell-show-do behavior management technique

Fig. 3. Modeling behavior management technique

The values for 3 different parameters (P1–P3) evaluated in the study were recorded on 4 occasions (T1–T4) for each group. The FLACC scores (P4) were recorded only during the intraoperative period (T3).

Dental procedure

After the intervention, the children in all 3 groups were prepared for the dental procedure. A class 1 or class 2 dental cavity preparation was carried out in primary/permanent molars. The surface was etched, rinsed, and a bonding agent was applied and cured. An appropriate shade of composite restoration was then placed and cured, and the occlusal adjustments were made. The entire procedure, from the initial stage of the cavity preparation to the placement of the restorative material and occlusal adjustment was completed in 20–30 min.

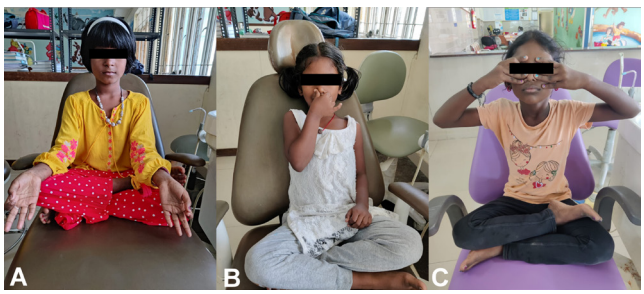

Fig. 4. Yogic relaxation behavior management technique
A. Pancha Kosha; B. Bhramari Pranayama; C. Nadi Shodhana.

Statistical analysis

Descriptive and inferential statistics were analyzed using the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA). The mean (*M*) and standard deviation (*SD*) were used to summarize the quantitative data. Multivariate analysis of variance (MANOVA) was employed to conduct intergroup comparisons of the 3 groups for the analyzed parameters (P1–P4) at various time points (T1–T4). A *p*-value of less than 0.05 was considered statistically significant.

Results

Preoperative period

The MANOVA test was conducted to examine the multivariate effects of the variable "group" on the dependent variables. The Pillai's trace test statistic yielded a significant result (F = 4.873, degrees of freedom (*df*) = 6.000, error *df* = 52.000, $p = 0.001$), indicating that there is a statistically significant multivariate effect of the "group" variable on the dependent variables. The effect size, as indicated by partial eta squared, was moderate (0.360). Additionally, the Wilks' lambda test statistic yielded a significant result (F = 5.351, *df* = 6.000, error *df* = 50.000, $p < 0.001$), providing additional evidence for the presence of a significant multivariate effect. The effect size was moderate (0.391). The Hotelling's trace test statistic was found to be significant (F = 5.807, *df* = 6.000, error *df* = 48.000, $p < 0.001$), providing further evidence of a significant multivariate effect, with a moderate effect size (0.421). Finally, the Roy's largest root test statistic yielded a highly significant result (F = 10.896, *df* = 3.000, error *df* = 26.000, $p < 0.001$), indicating a substantial multivariate effect of the "group" variable on the dependent variables. The effect size was large (0.557). Overall, the results of the MANOVA test demonstrate that the variable "group" has a significant and meaningful multivariate effect on the dependent variables (Table 1).

Table 2 shows the results of the between-subject effects analysis for the dependent variables "oxygen saturation", "pulse rate" and "FIS" in relation to the independent variable "group." With regard to the dependent variable "oxygen saturation", the Type III sum of squares was 44.067, and the mean square was 22.033. The F-value was 2.821, and the *p*-value was 0.077, which is not statistically significant. The effect size, as measured by partial eta squared, was 0.173, indicating a small effect. With regard to the pulse rate, the Type III sum of squares was 48.200, and the mean square was 24.100. The F-value was 0.817, and the *p*-value was 0.452, indicating that the observed effect is not statistically significant. The effect size was small (0.057). In contrast, for the dependent variable "FIS", the Type III sum of squares was 11.667, and the mean square was 5.833. The F-value was 13.938, and the *p*-value was <0.001, indicating a statistically significant effect. The effect size was considerable (0.508). In summary, the analysis demonstrates that the variable "group" has a significant influence on the dependent variable "FIS", with a large effect size. No statistically significant effects of the "group" variable on the oxygen saturation and pulse rate were identified, although a small effect size was observed for the "oxygen saturation" variable.

After conditioning

Table 3 presents the results of the multivariate test after conditioning, examining the effects of the variable "group" on the dependent variables. The Pillai's trace test statistic yielded a non-significant result (F = 0.585, *df* = 6.000, error *df* = 52.000, $p = 0.740$), suggesting that there is no statistically significant multivariate effect of the "group" variable on the dependent variables after conditioning. The effect size, as measured by partial eta squared, was 0.063,

Table 1. Results of the multivariate test in the preoperative period (T1)

Multivariate test							
effect		effect size	F	hypothesis *df*	error *df*	*p*-value	partial eta squared
Group	Pillai's trace	0.720	4.873	6.000	52.000	0.001*	0.360
	Wilks' lambda	0.371	5.351	6.000	50.000	<0.001*	0.391
	Hotelling's trace	1.452	5.807	6.000	48.000	<0.001*	0.421
	Roy's largest root	1.257	10.896	3.000	26.000	<0.001*	0.557

* statistically significant ($p < 0.05$, multivariate analysis of variance (MANOVA)); *df* – degrees of freedom.

Table 2. Results of the between-subject effects analysis in the preoperative period (T1)

Source	Dependent variable	Type III sum of squares	Mean square	F	*p*-value	Partial eta squared
Group	oxygen saturation	44.067	22.033	2.821	0.077	0.173
	pulse rate	48.200	24.100	0.817	0.452	0.057
	FIS	11.667	5.833	13.938	<0.001*	0.508

* statistically significant ($p < 0.05$, MANOVA); FIS – facial image scale.

indicating a small effect. Similarly, the Wilks' lambda test statistic yielded a non-significant result (F = 0.578, *df* = 6.000, error *df* = 50.000, *p* = 0.746), further confirming the abscence of a significant effect of the "group" variable on the dependent variables. The effect size was small (0.065). Additionally, the Hotelling's trace test statistic was found to be non-significant (F = 0.570, *df* = 6.000, error *df* = 48.000, *p* = 0.752), with a small effect size (0.066). Finally, the Roy's largest root test statistic also yielded a non-significant result (F = 1.162, *df* = 3.000, error *df* = 26.000, *p* = 0.343). The effect size was small (0.118). In summary, the results of the multivariate test after conditioning demonstrate that there is no statistically significant multivariate effect of the variable "group" on the dependent variables. The observed effect sizes are small, suggesting that the variable "group" has a minimal impact on the dependent variables after conditioning.

Intraoperative period

Table 4 presents the results of the multivariate test, indicating the effects of the variable "group" on the dependent variables in the intraoperative period. The Pillai's trace test statistic yielded a significant result (F = 3.913, *df* = 8.000, error *df* = 50.000, *p* = 0.001), suggesting a statistically significant multivariate effect. The effect size, as measured by partial eta squared, was moderate (0.385). Similarly, the Wilks' lambda test statistic yielded a significant result (F = 6.187, *df* = 8.000, error *df* = 48.000, *p* < 0.001), providing further evidence for the presence of a significant multivariate effect. The effect size was moderate (0.508). The Hotelling's trace test statistic was found to be statistically significant (F = 8.838, *df* = 8.000, error *df* = 46.000, *p* < 0.001), with a large effect size (0.606). Finally, the Roy's largest root test statistic yielded a highly significant result (F = 19.109, *df* = 4.000, error *df* = 25.000, *p* < 0.001), indicating a substantial multivariate effect of the variable "group" on the dependent variables. The effect size was large (0.754). In summary, the results of the multivariate test demonstrate that the variable "group" has a significant and meaningful multivariate effect on the dependent variables. The observed effect sizes are generally moderate to large, indicating a notable impact of the variable "group" on the dependent variables in the intraoperative period.

Table 5 presents the results of the between-subject effects analysis, examining the impact of the variable "group" on the dependent variables. With regard to the dependent variable "oxygen saturation", the Type III sum of squares was 76.067, while the mean square was 38.033. The F-value was 3.506, and the *p*-value was 0.044, indicating a statistically significant result. The effect size, as measured by partial eta squared, was moderate (0.206). Regarding the dependent variable "pulse rate", the Type III sum of squares was 447.200, and the mean square was 223.600. The F-value was 5.337, and the p-value was 0.011, which is statistically significant. The effect size was moderate (0.283). In relation to the FIS, the Type III sum of squares was 20.867, and the mean square was 10.433. The F-value was 8.668, and the *p*-value was 0.001, indicating a statistically significant effect. The effect size was large (0.391). Finally, for the dependent variable "FLACC", the Type III sum of squares was 81.867, and the mean square was 40.933. The F-value was 17.599, and the *p*-value was <0.001, indicating a highly significant effect. The effect size was large (0.566). In summary, the analysis reveals that the variable "group" has a considerable influence on the dependent variables. Significant effects were observed for all examined variables. The effect sizes were generally moderate to large, suggesting that the variable "group" has a notable impact on these dependent variables.

Table 3. Results of the multivariate test after conditioning (T2)

Multivariate test							
effect		effect size	F	hypothesis *df*	error *df*	*p*-value	partial eta squared
Group	Pillai's trace	0.127	0.585	6.000	52.000	0.740	0.063
	Wilks' lambda	0.874	0.578	6.000	50.000	0.746	0.065
	Hotelling's trace	0.142	0.570	6.000	48.000	0.752	0.066
	Roy's largest root	0.134	1.162	3.000	26.000	0.343	0.118

* statistically significant (*p* < 0.05, MANOVA).

Table 4. Results of the multivariate test in the intraoperative period (T3)

Multivariate test							
effect		effect size	F	hypothesis *df*	error *df*	*p*-value	partial eta squared
Group	Pillai's trace	0.770	3.913	8.000	50.000	0.001*	0.385
	Wilks' lambda	0.242	6.187	8.000	48.000	<0.001*	0.508
	Hotelling's trace	3.074	8.838	8.000	46.000	<0.001*	0.606
	Roy's largest root	3.057	19.109	4.000	25.000	<0.001*	0.754

* statistically significant (*p* < 0.05, MANOVA).

Postoperative period

Table 6 presents the results of the multivariate test, which examines the effects of the variable "group" on the dependent variables in the postoperative period. The Pillai's trace test statistic yielded a significant result (F = 4.824, *df* = 6.000, error *df* = 52.000, *p* = 0.001), indicating a statistically significant multivariate effect. The effect size, as measured by partial eta squared, was moderate (0.358). Similarly, the Wilks' lambda test statistic yielded a significant result (F = 6.612, *df* = 6.000, error *df* = 50.000, *p* < 0.001), further supporting the presence of a significant multivariate effect. The effect size was moderate (0.442). The Hotelling's trace test statistic was found to be significant (F = 8.531, *df* = 6.000, error *df* = 48.000, *p* < 0.001), with a moderate effect size (0.516). Finally, the Roy's largest root test statistic yielded a highly significant result (F = 18.137, *df* = 3.000, error *df* = 26.000, *p* < 0.001), indicating a substantial multivariate effect of the variable "group" on the dependent variables. The effect size was large (0.677). In conclusion, the results of the multivariate test demonstrate that the variable "group" has a significant and meaningful multivariate effect on the dependent variables. The observed effect sizes were generally moderate to large, indicating a notable impact of the variable "group" on the dependent variables.

Table 7 presents the results of the analysis for the between-subject effects, examining the effects of the variable "group" on the dependent variables. With regard to the dependent variable "oxygen saturation", the Type III sum of squares was 130.200, while the mean square was 65.100. The F-value was 4.929, and the *p*-value was 0.015, indicating a statistically significant result. The effect size, as measured by partial eta squared, was moderate (0.267). Regarding the dependent variable "pulse rate", the Type III sum of squares was 534.067, and the mean square was 267.033. The F-value was 4.575, and the *p*-value was 0.019, indicating a statistically significant effect. The effect size was considered moderate (0.253). With regard to the dependent variable "FIS", the Type III sum of squares was 34.200, and the mean square was 17.100. The F-value was 13.383, and the *p*-value was <0.001, indicating a highly significant effect. The effect size was large (0.498). In summary, the analysis reveals that the variable "group" has a significant influence on the dependent variables. The effect sizes are generally moderate to large, suggesting that the variable "group" has a notable impact on these dependent variables.

Discussion

Dental anxiety originates from the sight of an anxious stimulus, which can be inculcated during childhood and subsequently propagated into adulthood. The dentist should address the root cause in order to provide

Table 5. Results of the between-subject effects analysis in the intraoperative period (T3)

Source	Dependent variable	Type III sum of squares	Mean square	F	*p*-value	Partial eta squared
Group	oxygen saturation	76.067	38.033	3.506	0.044*	0.206
	pulse rate	447.200	223.600	5.337	0.011*	0.283
	FIS	20.867	10.433	8.668	0.001*	0.391
	FLACC	81.867	40.933	17.599	<0.001*	0.566

* statistically significant (*p* < 0.05, MANOVA); FLACC – Face, Leg, Activity, Cry, and Consolability scale.

Table 6. Results of the multivariate test in the postoperative period (T4)

Multivariate test							
effect		effect size	F	hypothesis *df*	error *df*	*p*-value	partial eta squared
Group	Pillai's trace	0.715	4.824	6.000	52.000	0.001*	0.358
	Wilks' lambda	0.311	6.612	6.000	50.000	<0.001*	0.442
	Hotelling's trace	2.133	8.531	6.000	48.000	<0.001*	0.516
	Roy's largest root	2.093	18.137	3.000	26.000	<0.001*	0.677

* statistically significant (*p* < 0.05, MANOVA).

Table 7. Results of the between-subject effects analysis in the postoperative period (T4)

Source	Dependent variable	Type III sum of squares	Mean square	F	*p*-value	Partial eta squared
Group	oxygen saturation	130.200	65.100	4.929	0.015*	0.267
	pulse rate	534.067	267.033	4.575	0.019*	0.253
	FIS	34.200	17.100	13.383	<0.001*	0.498

* statistically significant (*p* < 0.05, MANOVA).

an uncompromised treatment.[15] A variety of methods have been employed for behavior management in children, including behavior shaping and positive reinforcement, tell-show-do, enhancing control, distraction, systemic desensitization, and modeling.[16] The United Nations General Assembly adopted June 21st as the International Day of Yoga.[17] The yogic relaxation technique represents a less invasive alternative behavior management technique due to its novel approach and ease of application.

The concrete operational stage of cognitive development is observed in children between the ages of 7 and 11. As the study required the patients to understand and reproduce various yogic relaxation techniques, the age group of 6–12 years was selected for the study.[18]

Based on the learning theory of behavior management, the tell-show-do technique prepares the child to overcome their fear. It reduces uncertainty, alleviates anticipatory anxiety, and facilitates patient education and behavior guidance.[19,20]

Modeling is based on learned experience and observation of the visual impulses, which can be conducted through a live model or delivered through videos.[21,22] Live modeling is an essential alternative to the tell-show-do technique. Using parents as a live model is a tangible technique in dental clinical practice.[23,24] The tell-show-do and live modeling techniques have been shown to reduce anxiety in pediatric patients.[23,25–28] Thus, these techniques have been selected as the standard comparison methods for the yogic relaxation technique.

Yoga is used as an alternative treatment modality in various fields of medicine, including oncology. It improves the psychological well-being in adult cancer patients.[29–32] In the pediatric population, yoga has been shown to mitigate the emotional and psychological challenges associated with chemotherapy and radiotherapy.[33,34]

Yoga, along with visual modeling and pedagogy in mass education has been demonstrated to enhance the learning capability of children with autism spectrum disorder (ASD) in tooth brushing.[35] It also enhances motor activity and imitation skills, [36] and improves attention and hyperactivity among ASD children.[37] Additionally, it has been shown to reduce stress, anxiety and depression,[38–40] and to improve attentional control and heart rate variability.[41,42] The practice of yoga and mindfulness improves the socio-emotional function and regulatory skills, such as behavioral self-regulation and executive function.[43] Yoga has an anxiolytic effect in the management of adult patients undergoing endodontic treatment.[44]

Yoga modulates breathing activity, balances the sympathetic and parasympathetic systems, reduces the workload on the heart and the oxygen requirement, decreases the pulse rate, increases oxygen saturation, and induces relaxation.[45]

The pulse rate can be considered a psychological marker of anticipatory anxiety.[46,47] Fear and anxiety are triggered by breathlessness, a dysfunctional respiratory control mechanism, hypoxia, and hypocapnia.[48] Stress can alter the respiratory rate pattern, influencing the oxygen saturation and partial pressure of carbon dioxide in the blood.[49] The FIS is a reliable method for evaluating children's dental anxiety.[50,51] The FLACC is accurate and sensitive in measuring procedure pain.[52,53] Hence, the abovementioned parameters were selected to assess the anxiety and pain levels during the course of the study.

In the study, during both the intraoperative and postoperative periods, oxygen saturation was significantly higher in the yogic relaxation technique group. Additionally, the yogic relaxation technique group exhibited lower pulse rates, FIS and FLACC scores compared to the tell-show-do and modeling technique groups. The yogic relaxation technique was implemented through the use of 3 different yoga poses; however, no subgroups were designated to assess the efficacy of individual poses. To the best of our knowledge, our study was the first to use a yogic relaxation technique as a behavior modification technique among pediatric patients in a dental setting.

Conclusions

The practice of yoga has a positive influence on the general health of the individual. Various behavior management techniques, including the tell-show-do technique, have been know to reduce dental anxiety in children. Our study has shown the anxiolytic effect of the yogic relaxation technique in comparison to the tell-show-do and modeling techniques. Thus, the yogic relaxation technique can be considered an alternative behavioral modification technique for reducing dental anxiety among pediatric patients.

Ethics approval and consent to participate

The study was approved by the ethical clearance board of Chettinad Academy of Research and Education, Chennai, India (approval No. IHEC-I/1302/22).

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Priyanka Balakrishnan https://orcid.org/0000-0002-7838-7992
Daya Srinivasan https://orcid.org/0000-0001-5453-4380
Senthil Eagappan https://orcid.org/0000-0003-2933-6272

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Original paper

Comparison of bond failure with resin-modified glass ionomer cement and visible light-cured composite bonding systems in orthodontic patients: A split-mouth randomized controlled trial

Hafsa Qabool[1,A–D], Jaweriyah Qabool[2,A,B,D,F], Rashna Hoshang Sukhia[1,A,C,E,F], Mubassar Fida[1,A,E,F]

[1] Department of Surgery, Aga Khan University Hospital, Karachi, Pakistan
[2] Department of Dentistry, Dow University of Health Sciences, Karachi, Pakistan

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):651–657

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

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on October 30, 2022
Reviewed on February 24, 2023
Accepted on April 3, 2023

Published online on October 3, 2024

Abstract

Background. Resin-modified glass ionomer cement (RMGIC) is considered a fluoride-releasing bonding agent.

Objectives. The aim of the study was to evaluate the rate of bracket bond failure with light-cured composite (LCC) and RMGIC, and to evaluate factors that contribute to the rate of bracket failure with both bonding agents.

Material and methods. A randomized controlled trial was conducted on a sample size of 33 patients. The patients were randomly allocated for bonding with visible LCC (control group) or RMGIC (intervention group) using the lottery method. The study was double-blinded. The rate of bracket bond failure was assessed after a follow-up of minimum 3 months and evaluated using the survival regression analysis, taking into account the effects of bonding agents and other factors influencing bracket bond failure.

Results. A total of 33 participants were recruited for the study, and 66 quadrants for the intervention and control groups were randomly selected and analyzed. The data was normally distributed and the mean age of the subjects was comparable between both bonding systems. The results of the regression analysis indicated that there was no statistically significant difference between the rate of bracket bond failure with RMGIC and LCC ($p = 0.081$). However, after analyzing the mean days of survival, it was found that bracket survival was negligibly low with RMGIC, with a mean of 216.00 ±133.72 days as compared to LCC, with a mean survival of 224.11 ±124.59 days. No adverse effects were observed during the course of the trial.

Conclusions. There was no difference in the rate of bracket bond failure between the intervention and control groups. The survival rate of brackets treated with RMGIC was found to be comparable to that of LCC, with a minimal difference.

Keywords: bonding agent, bond strength, bracket failure

Cite as
Qabool H, Qabool J, Sukhia RH, Fida M. Comparison of bond failure with resin-modified glass ionomer cement and visible light-cured composite bonding systems in orthodontic patients: A split-mouth randomized controlled trial. *Dent Med Probl.* 2024;61(5):651–657. doi:10.17219/dmp/162970

DOI
10.17219/dmp/162970

Introduction

The bonding of brackets to the tooth surface is a technique-sensitive procedure that requires proficient operator control.[1] The ideal orthodontic bracket bonding materials should have adequate shear bond strength to reduce the incidence of bracket breakage.[2,3] Factors that contribute to the loss of bond strength between the bracket and the tooth include mastication occlusal forces, orthodontic stresses exerted by the archwire, the oral environment, and, most importantly, the bonding technique.[4–6] The occurrence of repeated breakages not only increases the duration of treatment but also compromises the treatment outcomes. For every bracket breakage, there are approx. 15 days of expected treatment time.[7,8] Frequent bracket bond failure may be indicative of poor patient cooperation toward orthodontic treatment.[9,10]

To increase the shear bond strength and reduce the rate of bond failure, a range of bonding agents have been introduced, including composite, resin-modified cement and polyacid-modified composites (compomers).[11,12] Each bonding material is polymerized by a different curing mechanism.[13] Composite, which is either polymerized by a self-activated chemical reaction or visible blue light activation, is a widely used material for orthodontic bracket bonding.[14]

In recent years, light-cured composites (LCCs) have been rapidly replaced by chemical cure bonding systems.[15] The LCC system contains camphorquinone, which serves as an initiator and is triggered by visible blue light at 420–450 nm.[16] The advantages of LCCs include increased working time, easier manipulation and increased accuracy of bracket placement while bonding.[17] The disadvantages of light-cured bonding systems include harmful effects of visible blue light and increased armamentarium.[18]

Patients undergoing orthodontic treatment are usually at high risk of caries. Previous reports have proven that the fluoride-releasing properties of glass ionomer cements (GICs) decrease the risk and progression of dental caries.[10,13,16] Fluoride is discharged as a result of the reaction between polyacid and aluminosilicate glass. As the glass network breaks down, it releases Ca^{2+}, Al^{3+} and F ions.[6] Glass ionomer cements also absorb fluoride from toothpaste or mouthwash rinses and re-mineralize themselves, releasing fluoride continuously. This eventually results in a reduction in the incidence of caries and white spot lesions (WSLs) around the brackets in orthodontic patients.[19] After the introduction of GICs, researchers observed that their bond strength was significantly lower than that of conventional composite (CC) materials, as reported in both in vivo and in vitro studies.[11,12] This led to the development of resin-modified GIC (RMGIC) in 1997.[19,20] Resin-modified GIC has combined the strength of the resin bond to enamel and the fluoride-releasing ability of GICs. This makes it a favorable material with a reduced bracket failure rate and a reduced occurrence of demineralized WSLs (DWSLs).[20] Despite all the efforts, RMGIC has not gained popularity due to a lack of publications on the subject. A study by Gaworski et al. evaluated the bracket failure rate of RMGIC and self-cured composite and concluded that the bracket failure rate of RMGIC was 25%, as opposed to the 7.4% bracket failure rate of CC.[21] A study by Choo et al. found no difference in the bracket failure rate between the 2 agents.[12] Similarly, a single-arm randomized controlled trial by Hitmi et al. demonstrated a clinically acceptable bracket failure rate with RMGIC.[20]

A number of studies have compared the shear bond strength of GIC with various polymerization techniques of composite.[18–20] Glass ionomer cements are being used as restorative materials, luting agents, cavity liners, and sealants.[20] However, the shear bond strength of GIC is questionable when compared with composite for the bonding of brackets. A novel modification has recently been introduced, comprising a combination of glass ionomer particles and resin composite filler particles, with the objective of increasing its strength. Previous reports have indicated that RMGIC is not only less technique-sensitive than composite resins but also exhibits caries resistance and has an efficient bond strength.[21,22]

Previous studies have compared the shear bond strength of various bracket bonding techniques.[14,23] For example, Hegarty and Macfarlane found a 16% bracket breakage rate with RMGIC bonding systems and a 3% bond failure rate with LCC.[23] They also reported no significant difference in the rate of bond failure between RMGIC and visible LCC.[23]

Objectives

The aim of the study was to compare the rate of bond failures in RMGIC and visible LCC over a three-month period. The null hypothesis was that there was no significant difference in the rate of bond failure between RMGIC and visible LCC.

Material and methods

Trial design

A double-blind, split-mouth, randomized controlled trial was conducted after obtaining ethical approval from the Aga Khan University Hospital (AKUH) Ethical Review Committee (approval No. 2022-5282-23281). The protocol for this clinical trial was registered in the ClinicalTrials.gov database (registration No. NCT06602154). There was no deviation in the protocol after registration. After the acceptance of the protocol and the commencement of the trial, no alterations were made to the assessment of the outcome. The principles of Good Clinical Practice (GCP) and the Declaration of Helsinki were strictly followed throughout the course of the study. The PICOS model used for this clinical trial is as follows: the Participants were orthodontic

patients; the Intervention was RMGIC; the Control was LCC; the Outcome was bracket bond failure; and the Study design was a split-mouth randomized controlled trial.

Study sample

The enrollment of participants started in October 2020, and the last participant was enrolled in January 2022. The patients were recruited from an orthodontic clinic of the Aga Khan University Hospital in Karachi, Pakistan. In the study, we included all patients who expressed willingness to initiate orthodontic treatment with fixed appliance therapy and who were aged between 14 and 40 years and perceived to be compliant. All patients who were willing to participate in the study signed the consent form. The age range of patients who preferred fixed orthodontic mechanotherapy was considerable. Consequently, age was included as a stratification variable in the regression analysis, with the objective of eliminating age as a confounding factor. Patients with enamel surface defects, fluorosis, or syndromic conditions (e.g., hypodontia or microdontia) were excluded from the study. Similarly, patients at high risk of dental caries and those who chose ceramic brackets due to aesthetic concerns were also excluded.

Interventions

All patients included in the study were bonded with fixed 0.022" slot metal brackets (3M™ Unitek™; 3M, Diegem, Belgium) in accordance with the Roth prescription. The bonding of the brackets was performed after full-mouth scaling and polishing, using a direct bonding approach in a split-mouth design. The control and intervention groups were bonded on the opposite arches in the contralateral quadrants and vice versa. Etching was conducted with 37% phosphoric acid for 15 s in both bonding systems. The nickel titanium (NiTi) wires were used for leveling and alignment, beginning with 0.012" NiTi wires and progressing sequentially to 0.018" Stainless Steel (SS) wires. The procedure was concluded with the use of a 0.07 × 25" SS wire. All molars were sealed with GIC.

The control in our study was the gold standard LCC (Transbond XT Light Cure Adhesive; 3M). The intervention was light-cured with Riva, resin-reinforced glass ionomer restorative cement (HV capsules refill). All patients included in the study underwent bonding with both LCC and RMGIC in the contralateral quadrants of the opposing arches. All participants were given the same instructions regarding the care of their brackets and the necessity of avoiding the consumption of hard food items, especially using the front teeth, as well as the need to refrain from fiddling with brackets using the tongue or fingers. Patients were asked to attend orthodontic visits at three-week intervals. During these visits, any bracket breakages were recorded. The procedure was conducted over a three-month period.

Outcomes

The primary objective of this study was to compare the incidence of bracket bond failure in the contralateral quadrants, from the central incisor to the second premolars, in both arches, between the use of RMGIC and LCC. In our clinical practice, bracket bond failure for all orthodontic patients is recorded in well-maintained orthodontic record files. These files also contain other variables, including pre-treatment extraoral and intraoral photographs, findings from model casting, and cephalometric variables. After 3 months, the incidence of bracket bond failure was assessed based on the files of all included patients, without knowing the type of bonding agent used for bracket attachment. Additionally, the duration of bracket survival and the total number of breakages were documented.

The secondary objective of this study was to identify the factors that can contribute to bracket bond failure in both bonding systems. The dataset included data from the incisors to the premolars in all quadrants, but excluded data from the molars. In the assessment of outcomes, only first-time bracket breakage and the duration of bracket survival were recorded. In accordance with standard clinical practice, all orthodontic patients with bracket breakages underwent rebonding with LCC.

Sample size

The sample size was calculated using OpenEpi v. 3.01 sample size calculator (https://www.openepi.com/Menu/OE_Menu.htm), based on the findings of Hegarty and Macfarlane, who reported a bond failure percentage of 30% with light-cured adhesive and a risk ratio of 2.6 (1.7–3.9) for RMGIC as compared to resin-based LCC.[23] In order to maintain the above risk ratio at a 5% level of significance (α) and 80% power of study (1-β), a minimum of 33 subjects were required for this study (Fig. 1).

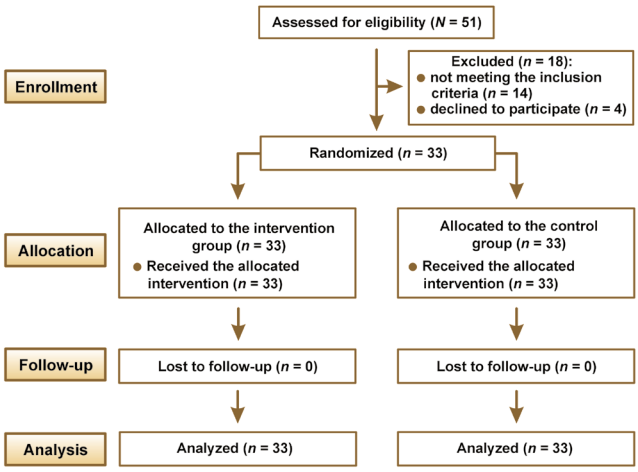

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram

Interim analyses and stopping guidelines

During the course of the study, no interim analysis was performed, and no adverse events were reported by any of the participants.

Randomization (sequence generation, allocation concealment, implementation)

Brackets were bonded in the contralateral quadrants by randomization. The upper right quadrant was bonded with RMGIC and LCC using a simple random lottery method, while the remaining quadrants were bonded in accordance with the split-mouth design. Patients were recruited, and the investigator (HQ) provided an overview of the study design and objectives.

Blinding

This trial was double-blinded. The patients were unaware of the bonding system used in each quadrant, as visible blue light was used to bond both RMGIC and LCC. The investigator responsible for recording the rate of bracket breakage from the patient files was also blinded.

Statistical analysis

The normality of the data was evaluated using the Shapiro–Wilk test on the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA). Subsequently, the data was analyzed using the Software for Statistics and Data Science (STATA, v. 12.0; StataCorp LCC, College Station, USA). Normally-distributed data was reported as means and standard deviations for continuous variables. All categorical variables were presented as frequency and percentages. The comparative analysis of the rate of bracket bond failure and the assessment of the factors influencing the bracket survival rate were conducted using the survival regression analysis.

Results

Participants flow

The participants were recruited from October 2020 to January 2022. A total of 33 participants were included in the study, and 66 quadrants were randomly bonded with each bonding system.

Baseline data

The descriptive variables, including age, sex, the divergence pattern, overjet, and overbite of the included patients are presented in Table 1.

Table 1. Descriptive statistics of the study participants

Parameter		Value
Sex *n* (%)	females	17 (51.5%)
	males	16 (48.5%)
Age [years] *M* ±*SD*		16.80 ±9.74
Divergence pattern *n* (%)	hypodivergent	10 (18.2%)
	normodivergent	17 (51.5%)
	hyperdivergent	6 (30.3%)
FMA [°] *M* ±*SD*		24.84 ±5.38
Overjet [mm] *M* ±*SD*		5.34 ±3.28
Overbite [mm] *M* ±*SD*		3.81 ±2.25

M – mean; *SD* – standard deviation; FMA – Frankfort-mandibular plane angle.

Numbers analyzed for each outcome, estimation and precision

The rate of bracket bond failure was assessed in 33 patients, with no follow-up loss reported during the course of this study. The stratification for bracket bond failure was conducted for both techniques based on the incisor relationship, vertical mandibular pattern, and the side and site of the jaw.

Comparison of bracket bond failure between the intervention and control groups

A demographics analysis revealed that the mean bracket survival rate for RMGIC was 216.00 ±133.72 days, while the gold standard LCC-bonded brackets survived for a mean duration of 224.11 ±124.59 days (Table 2). In this split-mouth randomized controlled trial, the incidence of bracket bond failure with RMGIC was found to be statistically non-significant ($p = 0.291$), with a hazard ratio of 1.44 when LCC was used as a reference.

Dentoalveolar factors influencing bracket bond failure

Using incisor class I as a reference, we found a non-significant difference in the incisor relationship of bracket bond failure in class II/1 ($p = 0.515$), class II/2 ($p = 0.060$) and class III ($p = 0.384$). Additionally, no significant difference was observed in bracket bond failure in the mandibular jaw when the maxillary jaw was used as a reference ($p = 0.462$). Similarly, no significant difference was noted in the posterior segment ($p = 0.163$) or the left site ($p = 0.110$) when the anterior segment or the right site, respectively, were used as references (Fig. 2). Moreover, overjet and overbite exhibited no statistically significant influence on bracket bond failure, with hazard ratios of 1.08 and 0.99, respectively (Table 3).

Table 2. Mean bracket survival for resin-modified glass ionomer cement (RMGIC) and light-cured composite (LCC)

Parameter		RMGIC (n = 33) [days]	LCC (n = 33) [days]
Incisor relationship	class I	187.38 ±113.57	197.71 ±117.76
	class II/1	218.40 ±145.14	226.98 ±129.90
	class II/2	256.16 ±89.71	226.98 ±129.90
	class III	246.80 ±129.87	267.11 ±124.90
Divergence pattern	hypodivergent	192.03 ±93.51	192.03 ±93.51
	normodivergent	210.10 ±141.40	210.10 ±141.40
	hyperdivergent	272.16 ±153.86	272.16 ±153.86
Segment	anterior	216.39 ±137.00	227.95 ±123.89
	posterior	215.40 ±129.17	218.37 ±125.88
Arch	maxillary	217.76 ±132.87	218.73 ±126.41
	mandibular	214.18 ±135.00	229.34 ±122.59
Site	right	217.76 ±132.87	270.23 ±132.02
	left	165.78 ±77.82	170.56 ±89.64
RU central incisor		167.26 ±89.05	276.66 ±136.06
RU lateral incisor		167.26 ±89.05	281.16 ±130.83
RU canine		161.73 ±94.60	273.83 ±134.41
RU first premolar		164.60 ±92.88	245.88 ±150.43
RU second premolar		167.26 ±89.05	236.88 ±141.75

Parameter	RMGIC (n = 33) [days]	LCC (n = 33) [days]
LU central incisor	260.00 ±191.94	167.26 ±89.05
LU lateral incisor	261.62 ±134.48	167.26 ±89.05
LU canine	281.16 ±130.83	167.26 ±89.05
LU first premolar	247.83 ±151.35	167.26 ±89.05
LU second premolar	255.88 ±136.19	159.733 ±93.72
RL central incisor	279.11 ±134.56	177.06 ±93.40
RL lateral incisor	259.00 ±189.79	176.56 ±93.72
RL canine	251.41 ±137.15	168.56 ±96.82
RL first premolar	279.11 ±134.56	176.56 ±93.72
RL second premolar	279.11 ±134.56	176.56 ±93.72
LL central incisor	167.26 ±89.05	269.11 ±125.82
LL lateral incisor	153.86 ±85.27	281.16 ±130.83
LL canine	137.60 ±92.22	281.16 ±130.83
LL first premolar	152.66 ±93.58	281.16 ±130.83
LL second premolar	145.73 ±93.14	275.33 ±124.59

RU – right upper; LU – left upper; RL – right lower; LL – left lower. Data expressed as *M* ±*SD*.

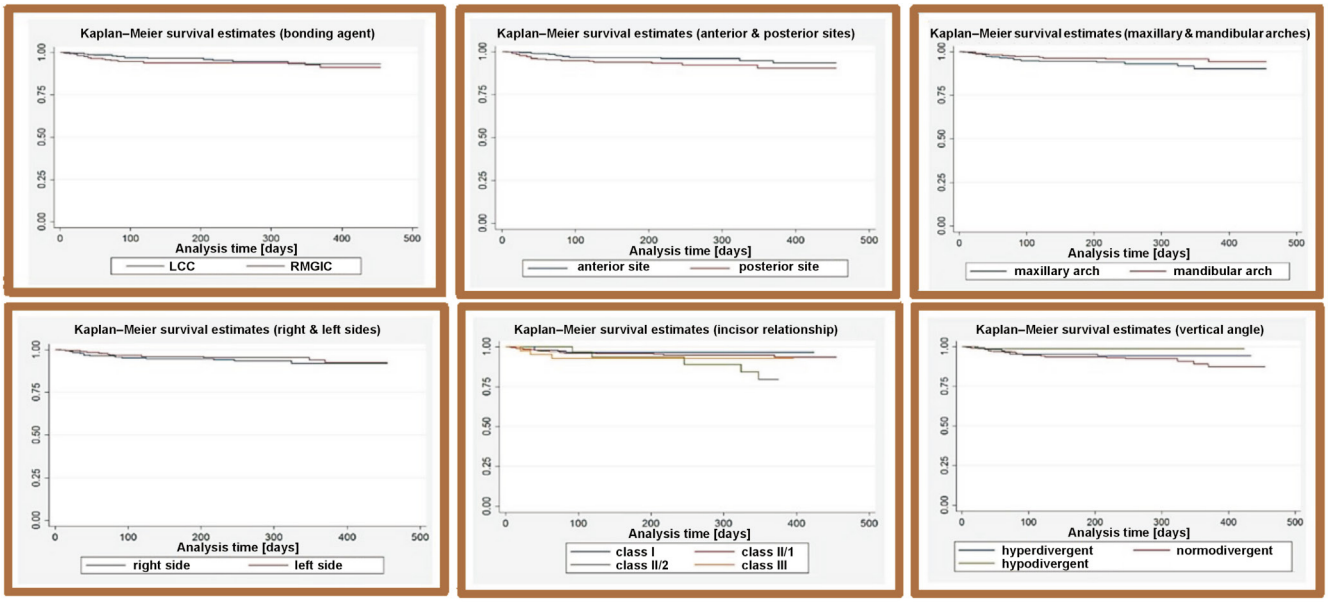

Fig. 2. Kaplan–Meier survival estimate graphs

Table 3. Factors influencing the survival of brackets

Variable		Hazard ratio	95% *CI*	*p*-value
Age		0.86	0.77–0.97	0.021*
Overjet		1.08	1.02–1.20	0.010*
Overbite		0.99	0.89–1.10	0.874
FMA		1.01	0.95–1.08	0.551
RMGIC (LCC used as a reference)		1.44	0.72–2.86	0.290
Divergent pattern (normodivergent pattern used as a reference)	hypodivergent	0.20	0.06–0.69	0.012*
	hyperdivergent	0.59	0.24–1.46	0.252
Mandibular jaw (maxillary jaw used as a reference)		0.77	0.39–1.53	0.465
Posterior segment (anterior segment used as a reference)		0.60	0.30–1.21	0.163
Left side (right side used as a reference)		1.73	0.88–3.40	0.103

* statistically significant ($p \leq 0.05$, survival regression analysis); *CI* – confidence interval.

Skeletal factors influencing bracket bond failure

In this study, we found that the hypodivergent profile had a statistically significant influence on bracket bond breakage (p = 0.020), with a hazard ratio of 0.20 when the normodivergent pattern was used as a reference. Conversely, the hyperdivergent profile demonstrated a non-significant influence (p = 0.251).

Adverse effects

No adverse effects were observed or reported during the course of the trial.

Discussion

In this split-mouth randomized controlled trial, no significant difference was observed in bracket bond failure between RMGIC and the gold standard LCC. Furthermore, our findings suggest that age and the hypodivergent profile significantly influence bracket bond failure in both bonding systems. In this study, we only assessed the first-time bracket bond failure for all participants who had been bonded with either RMGIC or LCC.

Available research on methods to reduce the prevalence and incidence of WSLs among orthodontic patients is still inconclusive, with significant discrepancies in the reported outcomes.[24–27] These discrepancies could be attributed to the lack of a gold standard method for the detection of demineralized spots on enamel surfaces.[26] Studies that employed clinical photographs for the comparison of WSLs with RMGIC and LCC exhibited no statistically significant difference over a three-month period.[24–26] However, Benson et al. reported that there was a significant difference in the severity of WSLs with RMGIC and LCC in the long-term follow-up.[24] Hence, in accordance with previous literature,[25–27] RMGIC should be used for bracket bonding in high caries-risk orthodontic patients to prevent further demineralization during treatment.

This study was designed to address the limitations of the available literature on the comparison and evaluation of bracket bond failure using RMGIC and LCC. Previous studies were limited by their comparison of similar outcomes between different subgroups of patients, which introduced numerous confounding factors, such as differences in oral hygiene maintenance, dietary patterns and habits, for all subjects.[24–26] Therefore, this study was designed as a split-mouth randomized controlled trial in which each participant was bonded with RMGIC as well as LCC.

Comparative ex vivo studies claimed that RMGIC forms a weak bond with the enamel surface compared to LCC.[27,28] This could be because the enamel tags do not form at an appropriate depth in dried extracted teeth. Consequently, this study was conducted among orthodontic patients to compare the incidence of bracket bond failure between the 2 bonding systems on the wet enamel surface of vital teeth. The GIC of RMGIC forms a chemical bond, whereas resin particles form a mechanical bond with the enamel tags.[20]

The findings of our study indicate that the bracket survival rate with RMGIC is clinically acceptable, thereby preventing the severity of enamel demineralization. Similar bond failure results with RMGIC were obtained in the studies conducted by Powis et al.[25] and Fricker.[26] We found that there was no significant difference in bracket bond failure between RMGIC and LCC. In contrast to these findings, Fricker's study reported a significant difference in bracket bond failure between the mandibular and maxillary jaws.[26] This discrepancy may be attributed to the fact that their study was not a split-mouth trial, with different patients allocated to receive RMGIC and LCC. Additionally, their results could be due to the difference in occlusal forces and deep bite in the studied population.

Moreover, researchers have claimed that light-cured RMGIC not only decreases the severity of WSLs but also has multiple clinical benefits, including fast setting time and a reduction in the inconvenience associated with primer application.[24–26] A study conducted by Kaup et al. claimed that light-cured RMGIC has greater bond strength compared to chemically-cured RMGIC.[27] Similarly, our study suggests that light-cured RMGIC should not only be clinically acceptable but also be the preferred bonding agent for orthodontic patients at high risk of caries.

The major strength of this trial is its split-mouth design. A recent study conducted by Qabool et al. described a new technique for the bonding of orthodontic brackets to reduce aerosol generation during the coronavirus disease 2019 (COVID-19) pandemic.[29] We propose that RMGIC and LCC should also be compared with this new technique. However, to generate strong clinical evidence, further clinical trials with larger sample sizes are required. Furthermore, future studies should be conducted with long-term follow-up to evaluate the first evidence of WSLs.

Conclusions

The results of this randomized controlled trial indicate that there is no statistically significant difference in the incidence of bracket bond failure between light-cured RMGIC and LCC. The rate of bracket survival with RMGIC is clinically acceptable to justify its use in orthodontic patients at high risk of dental caries.

Ethics approval and consent to participate

The study was approved by the Aga Khan University Hospital (AKUH) Ethical Review Committee (approval No. 2022-5282-23281). All patients who were willing to participate in the study signed the consent form.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Hafsa Qabool https://orcid.org/0000-0002-8968-2014
Jaweriyah Qabool https://orcid.org/0000-0001-7308-264X
Rashna Hoshang Sukhia https://orcid.org/0000-0001-9210-6432
Mubassar Fida https://orcid.org/0000-0003-4842-9896

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Original paper

Kinesio Taping as an alternative therapy for limited mandibular mobility with pain in female patients with temporomandibular disorders: A randomized controlled trial

Magdalena Gębska[1,A–F], Bartosz Dalewski[2,D–F], Łukasz Pałka[3,D–F], Paweł Kiczmer[4,B,C,F], Łukasz Kołodziej[1,A,E,F]

[1] Department of Rehabilitation Musculoskeletal System, Pomeranian Medical University, Szczecin, Poland
[2] Department of Dental Prosthetics, Pomeranian Medical University, Szczecin, Poland
[3] Private Dental Practice, Żary, Poland
[4] Department and Chair of Pathomorphology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Zabrze, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):659–670

Address for correspondence
Łukasz Pałka
E-mail: dr.lpalka@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on August 9, 2023
Reviewed on September 20, 2023
Accepted on October 1, 2023

Published online on June 4, 2024

Cite as
Gębska M, Dalewski B, Pałka Ł, Kiczmer P, Kołodziej Ł. Kinesio Taping as an alternative therapy for limited mandibular mobility with pain in female patients with temporomandibular disorders: A randomized controlled trial. *Dent Med Probl.* 2024;61(5):659–670. doi:10.17219/dmp/173126

DOI
10.17219/dmp/173126

Abstract

Background. Kinesio Taping (KT) is a non-invasive therapy commonly used in physiotherapy (PT). However, the available data on its effectiveness in patients with symptomatic temporomandibular disorders (TMD) remains scarce and contradictory.

Objectives. The aim of the study was to evaluate the analgesic and myorelaxant effects of KT in TMD patients with limited mandibular mobility.

Material and methods. A single-blind randomized controlled trial was conducted among female patients aged 20–45 years with Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) group Ib, using a parallel group design and equal randomization (1:1). All patients underwent surface electromyography (sEMG) of the masseter muscle (MAS), pain intensity was assessed using a Numeric Rating Scale (NRS), and temporomandibular joint mobility was measured before and after 6 and 12 days of treatment. The Perceived Stress Scale (PSS-10) questionnaire was administered on the first and last days of treatment. Statistical analysis was performed using analysis of variance (ANOVA). Mauchly's sphericity test determined changes over time and between groups for variables with a normal distribution. Bonferroni's correction was used for post hoc multiple comparisons. Variables with a non-normal distribution were analyzed using the nparLD package and multiple comparison post hoc test, while correlations were assessed using Spearman's coefficient.

Results. Each treatment had a significant effect on the bioelectrical sEMG parameters ($p = 0.05$). Kinesio Taping had a superior analgesic effect compared to the controls ($p < 0.001$). The combination of KT with therapeutic exercise (TE) proved to be a more effective therapy for improving the maximal mouth opening (MMO) and reducing perceived stress than monotherapy ($p < 0.001$). Minimally significant clinical differences were observed for sEMG, MMO and PSS-10 parameters after both therapies.

Conclusions. Kinesio Taping combined with TE may be considered an effective complementary non-invasive treatment modality for TMD, either as a stand-alone or as part of the therapeutic process in patients experiencing pain and limited mandibular ROM. Additionally, the use of KT and TE was found to have a beneficial effect on perceived stress levels.

Keywords: physiotherapy, Kinesio Taping, electromyography, temporomandibular joint, orofacial pain

Introduction

The etiology of temporomandibular disorders (TMD) is considered multifactorial and continues to be under increased scrutiny by researchers and orofacial pain clinicians. Various factors, including stress, genetic determinants, occlusal factors, and environmental factors (especially psychoemotional and psychosocial factors), have been identified as common causes of TMD.[1–3] The clinical manifestations of TMD can be both subjective and objective. The most common symptoms include decreased mandibular range of motion (ROM), pain in the masticatory muscles and/or temporomandibular joints (TMJs), joint clicking, tinnitus, pre-auricular pain, headaches and/or cervical spine pain, and increased head and neck muscle tension.[4,5] According to contemporary scientific findings, chronic myofascial pain accounts for more than 50% of all TMD diagnoses,[6] and the prevalence of TMD is estimated to be greater than 5% of the population.[6] Lipton et al. showed that approx. 6–12% of evaluated patients had 1 or more TMD symptoms.[7] Wieckiewicz et al. conducted research on the Polish population and found that 48.8% of patients were diagnosed with TMJ disorders, with displacement of the intervertebral disc with reduction being the most common (47.9%).[8] The prevalence of TMD patients is highest among those aged between 20 and 40,[9] and women are affected 1.5–2.5 times more often than men.[10]

The latest TMD standards of care highlight the importance of an individualized and multidisciplinary approach to establish a diagnosis and implement treatment as early as possible. This involves the prevention of chronic pain via local sensitization.[11] The patient should be referred early to a therapeutic team, consisting of a dentist with a background in restorative, prosthetics, or orofacial pain, a psychologist and/or psychiatrist if necessary (therapy of psychoemotional disorders of multiple etiology), a physiotherapist (pain reduction, restoration of normal TMJs and cervical spine biomechanics and ROM, and retraining of muscle engrams), and other specialists, based on the reported symptoms, e.g., neurologist, rheumatologist, or ear, nose and throat (ENT) specialist.[12,13]

Numerous studies emphasize the important role of physiotherapy (PT) in the recovery of patients with TMD.[14] Therapeutic modalities that enhance the effects of treatment are increasingly well documented and include physical therapies such as transcutaneous electrical nerve stimulation (TENS) and lasers, manual therapy (manipulation and mobilization, and soft tissue treatments), and therapeutic exercise (TE).[15] A prevalent method in musculoskeletal rehabilitation that has recently been implemented in dentistry is Kinesio Taping (KT).

Kinesio Taping (or elastic therapeutic taping) was developed by Dr. Kenzo Kase in the 1970s as a therapeutic method.[16] The process involves attaching cotton elastic bands to the skin's surface using a hypoallergenic acrylic adhesive.[16] The patches are applied with an initial tension (paper-off tension) of 10–25% and a maximum elasticity of 130–140% of the original length.[17] The tape parameters were designed to mimic human skin and achieve stretchability, greater mobility and adhesion. However, there is limited evidence from peer-reviewed studies on KT. The majority of knowledge on this topic is based on case studies, which may not be sufficient to support its use in contemporary evidence-based medicine. In theory, KT has high therapeutic potential and is thought to facilitate or inhibit muscle function, enable pain-free ROM, improve proprioception, relieve pain, optimize joint alignment, and reduce swelling. Moreover, KT speeds up the healing process and reduces recovery time by decreasing inflammation and pain, increasing blood flow and facilitating neurological rehabilitation.[18–21]

Although KT is widely used in clinical practice, its mechanism of action has not been fully understood.[22] The muscle-fascia chain tension segregation theory, in particular, is the most widely accepted principle.[22] When applied to the skin, KT causes microcoils that increase the subcutaneous space, improve lymphatic fluid and blood flow in the affected area, and stimulate the healing process in the damaged tissues over time.[23] This lays the foundation for the healing process established by KT. The process involves several modalities, such as relieving pressure on the underlying painful or sensitized tissue, creating space for lymphatic fluid movement, improving blood flow, and reducing pain by decreasing pressure on nociceptors.[23] In addition, by modulating muscle tone and stimulating cutaneous receptors, it is possible to restore the complex myofascial function, leading to improved proprioception and increased recruitment of muscle motor units.[24–26] The pain-alleviating effect of KT is believed to be due to the microscopic lifting effect of the skin, which improves lymphatic and blood circulation through stimulation of sensory pathways. Consequently, this process may increase afferent feedback and relieve sensory receptor irritation.[27,28] The effect of dynamic patching also improves self-esteem, as evidenced by numerous studies that highlighted the impact of KT on the patient's psychoemotional state.[29]

Kinesio Taping can be used alone or in combination with other methods to enhance the therapeutic efficacy. Although it was initially used in the field of sports medicine with surprisingly good outcomes, KT is often insufficient to obtain the expected result and is therefore rarely used as a monotherapy in TMD.[30] Currently, its use is more widespread, with KT being employed in various fields of medicine, including orthopedics, traumatology, surgery, neurology, oncology, gynecology, and pediatrics.[17,31]

The annual increase in the number of TMD patients is pushing clinicians to seek new, faster, alternative, and more efficient approaches to managing pain, elevated muscle tension and reduced TMJ mobility. Scientific

reports in the field of dentistry indicate that KT is a method used to eliminate pain, particularly within the musculoskeletal system.[32,33] Currently, KT is increasingly used in dental and maxillofacial surgery as a method to assist in postoperative treatment.[34,35] However, a few randomized trials on the efficacy of KT in TMD patients indicate a need to expand this knowledge.[36] Therefore, our goal was to evaluate additional effects, if any, of KT compared to the standard treatment regimen of counseling and self-therapy in TMD patients. We hypothesized that KT will provide additional improvements in pain intensity, changes in bioelectric muscle function, and improved functional mobility in TMD. The use of KT may affect the emotional state of TMD patients, leading to an improvement in well-being by reducing stress levels.

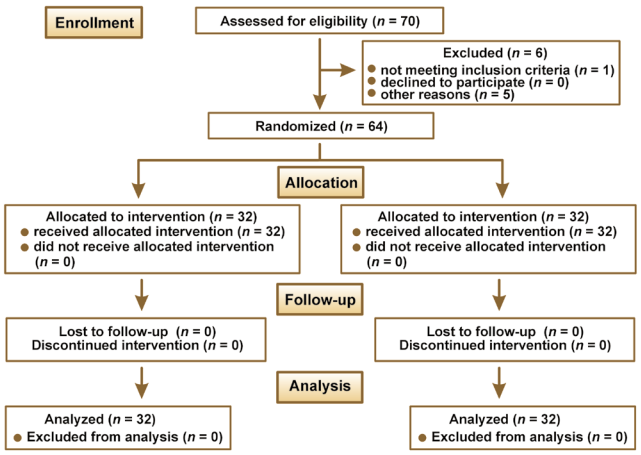

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT)[37] flowchart of the participants' progress through the trial phases

Material and methods

Trial design

This parallel, two-arm, randomized controlled trial with an equal allocation ratio (1:1) followed the Consolidated Standards of Reporting Trials (CONSORT).[37] The study was conducted at the University Dental Clinic of the Pomeranian Medical University in Szczecin, Poland. Participants who met the inclusion criteria were recruited between October 2022 and January 2023. Individuals attended clinic appointments at the time of randomization (baseline), as well as at 6- and 12-day intervals from baseline. Patients were divided into 2 groups in which PT was carried out for 12 weeks (excluding Saturdays and Sundays). All interventions in both study groups were performed free of charge, under the same conditions and by the same physiotherapist. Figure 1 depicts a flowchart of the participants' progress through the trial phases, in accordance with the CONSORT criteria.

The study was approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin (approval No. KB - 0012/102/13). The trial was registered in the ClinicalTrials.gov database (registration No. NCT05021874).

Participants

The study included 64 women (N = 64) between the ages of 20 and 45 years who were diagnosed with myofascial pain with mouth opening restriction for more than 3 months according to the Diagnostic Criteria for TMD (DC/TMD) group Ib. Patients were randomly assigned (simple randomization) to the experimental group (KTG, n = 32, standard deviation (SD) = 9.34) or the control group (CG, n = 32, SD = 8.2).

Study exclusion criteria were inflammation in the oral cavity manifested as myospasm or preventive muscle contraction, previous splint therapy, pharmacotherapy (e.g., oral contraception, hormone replacement therapy and antidepressants), systemic diseases (e.g., rheumatic and metabolic diseases), mental illness, lack of orthopedic stability of the mandible, masticatory organ or whiplash injury, pregnancy, patients undergoing orthodontic treatment, other types of inflammation in the oral cavity (e.g., pulpitis or impacted molars), and fibromyalgia or dermatologic disease.

All women underwent intraoral and extraoral dental examination by a dentist specialized in orofacial pain. The aim was to rule out odontogenic, periodontal and intracapsular origins of TMD pain.

A dentist determined whether the patient met the inclusion criteria based on the patient's history and physical examination. Another dentist was involved in the randomization of the patients.

The individuals qualified for the study underwent instrumental diagnostics, including surface electromyography (sEMG) of the masseter muscle (MAS) at rest and during exercise, and linear measurement of the range of maximal mouth opening (MMO). The intensity of pain was assessed on the Numeric Rating Scale (NRS). These measurements were then performed after the 6th and 12th days of treatment. The Perceived Stress Scale (PSS-10) questionnaire was used to assess the perceived stress levels of all subjects before and after the 12th day of therapy.[38]

Interventions

The KTG received MAS KT, counseling and TE. The CG received counseling and TE. After the 6th and 12th days of treatment, all patients were assessed for MAS sEMG, mandibular ROM and NRS.

During the entire treatment process, most attention was paid to patient cooperation. Therefore, lifestyle counseling and instructions for TE were initially implemented in all subjects (Table 1). Patients were informed about the causes of their dysfunction and how they could self-control their occlusal and non-occlusal oral habits, especially

teeth clenching, grinding, gum chewing, and nail biting. The participants were also informed about parafunctional self-management, the pathophysiology of the potential dysfunction, and the influence of their therapeutic interaction on the effectiveness of treatment. A standardized exercise regimen was presented to all subjects, with the application of KT also implemented in the KTG.

Therapeutic exercises (self-therapy)

Each participant was given a paper TE program by the physiotherapist. The program included a description of each exercise and instructions for performing them daily (frequency: 6 times a day, 10 repetitions each) throughout the study period (Table 1).[39]

Application of Kinesio Taping in the experimental group

Certified hypoallergenic 5-cm-wide tapes (K-Active; Nitto Denko Corporation, Osaka, Japan) were used for the study. Before the application of the tape, the patient's skin was cleaned twice with an alcohol-based solution and dried with a paper towel. The KT application was performed by a qualified physiotherapist. The tape was applied over the MAS while the patient was seated with their back stabilized against the back of a chair. The taping technique followed that of Benlidayi et al. for TMJs.[40]

The length of the tape was measured for each patient individually, from the preauricular region to the corner of the nose. The patient was instructed not to clench their teeth or make tooth contact and to relax their facial and neck muscles during the application process. To apply the tape, a Y-shaped patch was used. The therapist warmed the patch by rubbing it 3 times in the palm of their hands to activate the adhesive. Subsequently, the therapist asked the patient to open and close their mouth twice to palpate the TMJs. The bottom (base) of the tape was adhered to the TMJ area with no tension (0%). Then, the upper and lower branches of the patch were attached with approx. 10–15% tension.[40] The application was carried out on the right and left sides of the face (Fig. 2).

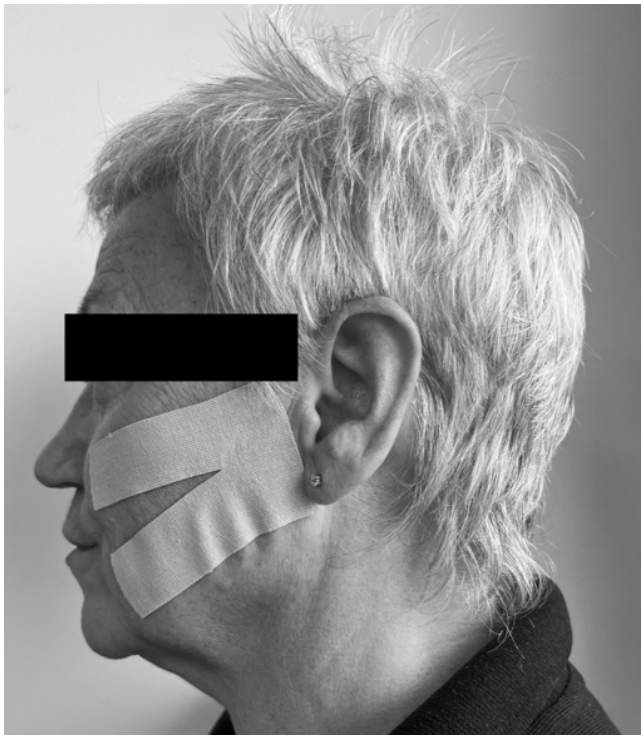

Fig. 2. Application of Kinesio Taping (KT) (own source)

Primary outcome measures

Pain severity scale

The severity of pain was assessed using the NRS in both the KTG and CG after each therapy session. The NRS is a tool used to measure pain intensity on a scale from 0 to 10, with 0 indicating no pain and 10 indicating the worst possible pain. Additionally, mandibular ROM was assessed. Pain intensity was measured during the preliminary examination, as well as after the 6th and 12th days of treatment.

Surface electromyography of the masseter muscle

All patients were assessed for MAS sEMG during the preliminary examination and after the 6th and 12th days

Table 1. Therapeutic exercises in the control (CG) and experimental (KTG) groups

Therapeutic exercise	Starting position	Movement
Gerry's exercise	tongue positioned on the palate	slow movements of opening and closing the mouth
Active exercises for lateral movements of the mandible	maxillary and mandibular teeth separated by about 5 mm	a slow motion of the mandible to the left, back to the midline, to the right, and again back to the midline
Side-to-side exercise	holding the front of the pen or pencil between upper and lower teeth	a slow motion of the lower jaw from side to side
Protrusion and mouth opening	teeth separated	lowering the lower jaw forward, opening the mouth, closing the mouth, retracting the lower jaw
Self-massage of the masseter muscle	teeth separated, hands clenched into a fist, placed around the mandibular branch (right hand on the right side, left hand on the left side)	circular rubbing movements of the masseter muscle with a pressure of approx. 0.5 kg
Cervical spine exercise (active flexion and extension movements of the spine)	standing or sitting with the head in a neutral position (gazing straight ahead)	bending the head forward and returning to the starting position, straightening the head and returning to the starting position

of treatment. The sEMG recordings were obtained in the morning hours, and the patients were instructed to refrain from drinking coffee, tea, or other stimulants before signal acquisition.

The study utilized a two-channel electromyograph (NeuroTrac® MyoPlus 2; Verity Medical Ltd., Tagoat, Ireland) with NeuroTrac® software (Verity Medical Ltd.) in clinical mode to record MAS sEMG activity. To ensure precise sEMG measurements, a band-stop filter was employed to eliminate interference from frequencies of 50 Hz and 60 Hz (mains) during recording (measured in microvolts [μV]). The application of specialized filtering enables the acquisition of sEMG measurements with a precision of 0.1 μV.

To prevent magnetic interference during sEMG measurements, the device was positioned at a distance of at least 4 m from cell phones or other potential sources of interference. The test was conducted using 2 unipolar electrodes, which were placed 10 mm apart. The electrodes were positioned over the center of the muscle body, parallel to the path of its fibers. The lower electrode was approx. 5 mm above the mandibular angle, while the upper electrode was 10 mm above it. The placement of the electrodes was preceded by careful palpation of each muscle by an experienced clinician to identify the thickest part of the muscle body. The bioelectrical signals of the MAS were acquired while the subject was seated upright with the head in a natural position, hands resting on the knees, and feet on the ground. Before the application of the electrodes, the skin was cleaned with rubbing alcohol, following the manufacturer's recommendations and Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles (SENIAM) guidelines (www.seniam.org). The ground electrode was placed on top of the C7 styloid process in the cervical section. This area is usually devoid of vastly active muscle fibers and is considered the optimal location for ground electrode placement in the orofacial region. This placement prevents cross-talk from muscles and electrodes that are not relevant to the examiner.

Electrical activity of the masseter muscle at rest

A rest test (RLX) was conducted on patients, with their dental arches slightly open and their tongues in a resting position.[41] Patients were instructed not to swallow saliva during the examination. Three measurements were taken 3 times, and the mean value was calculated.

Bioelectrical activity of the masseter muscle during maximal voluntary contraction

The sEMG signal was recorded in a sitting position with the teeth clenched with the greatest possible force for 5 s. The computer program connected to the device registered the minimum and maximum values and calculated the mean electric potentials, which, in conjunction with signal standardization, are considered essential for providing repeatable and unbiased electric signal acquisition.[41] Three measurements were taken, each recorded 3 times, and the mean value was calculated. The sEMG values were normalized by calculating the ratio of RLX to maximal voluntary contraction (MVC) using the following formula (Equation 1)[42]:

$$\%MVC\ [\%] = RLX\ [\mu V]/MVC\ [\mu V] \times 100\%. \quad (1)$$

Secondary outcome measures

Perceived Stress Scale

Perceived stress levels were assessed in the KTG and CG during the preliminary examination and after the 12th day of therapy. The examination used the standard paper-and-pencil method, with the patient seated at a table and no individuals in their immediate vicinity to avoid any influence on the responses. The study was conducted in a moderately dampened room with a pre-set air temperature of 22°C and without any time constraints.

The PSS-10 contains 10 questions about different subjective feelings related to personal problems and events, behaviors, and coping mechanisms. Respondents provide answers by selecting a number from 0 to 4 (0 – never, 1 – almost never, 2 – sometimes, 3 – quite often, 4 – very often). The overall score is the sum of all points, ranging from 0 to 40. The higher the score, the greater the perceived stress severity. The sten score properties determine the interpretation of the general indicator after conversion to standardized units. Scores ranging from 1 to 4 are considered low, while those ranging from 7 to 10 are considered high. Scores between 5 and 6 are considered average.[38]

Sample size

The sample size of 44 was determined for repeated measures analysis of variance (RM-ANOVA) with within-between interactions using the effect size of 0.25, α of 0.05, and power of 0.95.[43]

Randomization and blinding

Patients were assigned to the study group using simple randomization with the opaque closed envelope method. Allocation (1:1) concealment was achieved through consecutively numbered sealed envelopes, which were carefully checked to ensure they were undamaged and not see-through when held against a light source. Randomization was carried out by an investigator who was not

involved in the determination of patient eligibility, intervention delivery or data collection.

The outcome assessors were blinded to the group allocation and were not involved in providing the interventions (single-blind). The statisticians who conducted the statistical analyses were also blinded to the group allocation until after the analyses were completed. There was no need to unblind any of the participants at any point during the study.

Statistical analysis

The normality of the variables was assessed using the Shapiro–Wilk test and Q–Q plots. For variables with a normal distribution, ANOVA and Mauchly's sphericity test were used to determine changes over time and between groups. The Greenhouse–Geisser correction was applied if the assumption was not met, and Bonferroni's correction was used for post hoc multiple comparisons. Variables with a non-normal distribution were analyzed using the nparLD package and a multiple comparison post hoc test. The minimal important difference (*MID*) value was calculated as 1/2 of the *SD* of each parameter's initial value. The differences between the parameters were calculated by subtracting the final value from the initial value and then correlating these values using Spearman's coefficient. The level of significance set for the study was set at $p < 0.05$. The analysis was conducted using the R Studio software (Posit, Boston, USA; https://posit.co).[44] Wilcoxon test was performed to assess differences between the study and control participants.

Results

Baseline data

Table 2 presents the results of the preliminary study for the KTG and CG. In the preliminary examination, no significant differences were found between the groups regarding age, sEMG MVC, sEMG %MVC, MMO, left lateral movement (LLM), and the PSS-10.

Primary analysis

The primary analysis was conducted based on an intention-to-treat principle and included 64 patients who were randomly assigned. The patients were analyzed according to the protocol.

Table 2. Statistical analysis for age, surface electromyography (sEMG) values, temporomandibular joint (TMJ) range of motion (ROM), and pain intensity in the control group (CG) and the experimental group (KTG) at baseline

Variable	Group	Min	Max	*Me*	*SD*	*CI*	Q1	Q3	*p*-value
Age [years]	CG	20	45	29	8.2	2.82	25.5	39.5	0.994
	KTG	20	45	30	9.34	3.49	23.5	42	
sEMG RLX [μV]	CG	4.38	17.8	9.19	3.31	1.14	7.54	12.9	0.020*
	KTG	4.78	20	10.5	3.24	1.21	8.74	12.3	
sEMG MVC [μV]	CG	161	484	284	92.2	31.7	221	344	0.738
	KTG	137	438	275	68.8	25.7	238	331	
sEMG %MVC [%]	CG	19.6	31.8	25.6	3.21	1.1	23	28	0.192
	KTG	18.3	33.3	28.3	4.2	1.57	25.2	30.7	
NRS	CG	5	8	6	0.891	0.306	5	6.5	0.003*
	KTG	5	8	7	0.884	0.33	6	7	
MMO [mm]	CG	33	40	37	1.4	0.481	36	37.5	0.082
	KTG	31	38	36	1.53	0.572	35	37	
LLM [mm]	CG	5	7	6	0.657	0.226	5	6	0.077
	KTG	5	8	6	0.986	0.368	5	7	
RLM [mm]	CG	5	7	6	0.684	0.235	5.5	6	0.001*
	KTG	5	9	6	0.952	0.356	6	7	
PSS-10 [stens]	CG	5	10	8	1.36	0.466	7	9	0.288
	KTG	5	10	8	1.56	0.583	7	9	
PSS-10 [points]	CG	15	31	23	4.5	1.55	20	27	0.227
	KTG	14	35	25.5	5.83	2.18	20.2	28.8	

RLX – rest test; MVC – maximum voluntary contraction; %MVC – ratio of RLX to MVC; NRS – Numeric Rating Scale; MMO – maximal mouth opening; LLM – left lateral movement; RLM – right lateral movement; PSS-10 – Perceived Stress Scale; Min – minimum; Max – maximum; *Me* – median; *SD* – standard deviation; *CI* – confidence interval; Q1 – first quartile; Q3 – third quartile; * statistically significant ($p < 0.05$, Wilcoxon test).

Outcomes and estimations

The results of the statistical analysis of MAS bioelectrical activity before treatment (1), after 6 days of treatment (2) and after 12 days of treatment (3) in the KTG and CG are presented in Fig. 3.

Both the KTG and CG showed differences in the sEMG parameters (RLX, MVC, %MVC) of the MAS over time, indicating that each treatment affected the bioelectrical signal of the muscle ($p < 0.05$) (Fig. 3). No significant differences were observed between the groups when comparing the applied treatments after 6 days (RLX: $p = 0.192$; MVC: $p = 0.555$; %MVC: $p = 0.246$) and 12 days of treatment (RLX: $p = 0.430$; MVC: $p = 0.334$; %MVC: $p = 0.318$).

Table 3 shows the 95% confidence intervals (*CI*s) for sEMG after 6 and 12 days of treatment in the KTG and CG.

Figure 4 presents the results of the mandibular mobility analysis (LLM, right lateral movement (RLM), MMO) before treatment (1), after 6 days of treatment (2) and after 12 days of treatment (3) in the KTG and CG.

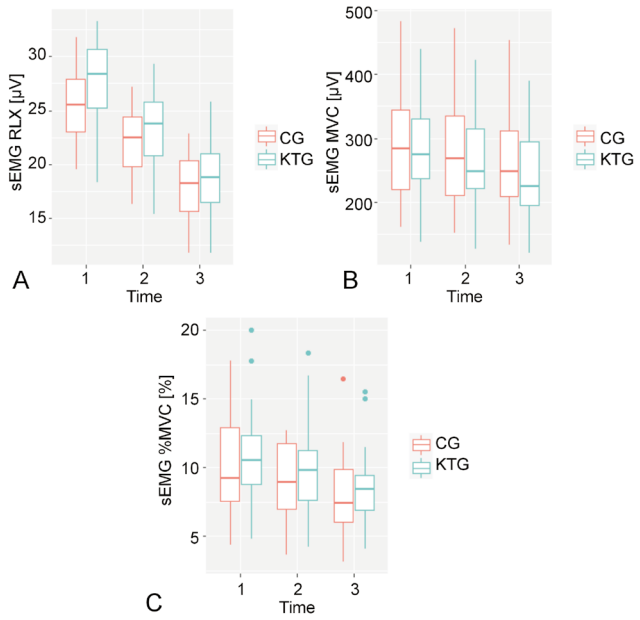

Fig. 3. Statistical analysis of the surface electromyography (sEMG) of the masseter muscle (MAS) in the control (CG) and experimental (KTG) groups before treatment (1), after 6 days of treatment (2) and after 12 days of treatment (3)

A. Rest test (RLX); B. Maximal voluntary contraction (MVC); C. The ratio of RLX to MVC (%MVC).

Table 3. 95% confidence intervals (*CI*s) for sEMG after 6 and 12 days of treatment in the CG and KTG

Variable		After day 6		After day 12	
		CG	KTG	CG	KTG
sEMG	RLX [μV]	23.0–28.8	21.62–24.58	16.86–18.94	17.27–19.93
	MVC [μV]	244.8–305.2	237.5–288.5	228.5–287.5	215.8–264.2
	%MVC [%]	7.916–9.884	8.62–10.92	8.86–8.80	7.546–9.474

Data presented as 95% *CI*.

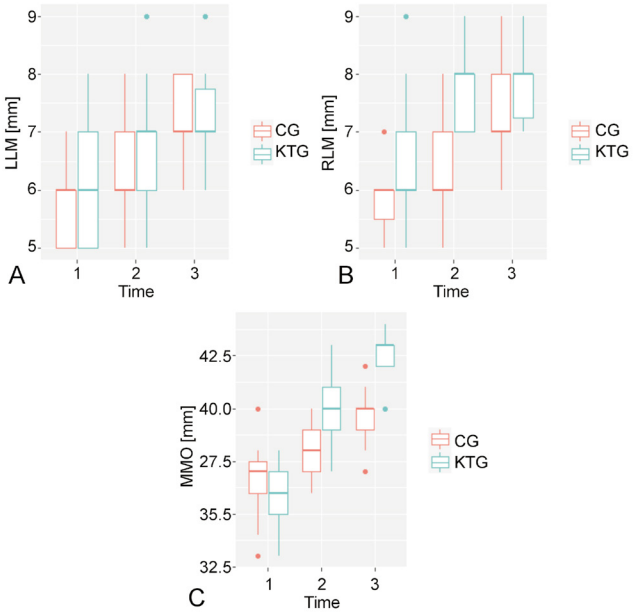

Fig. 4. Statistical analysis of the mandibular mobility range in the KTG and CG before treatment (1), after 6 days of treatment (2) and after 12 days of treatment (3)

A. Left lateral movement (LLM); B. Right lateral movement (RLM); C. Maximal mouth opening (MMO).

The parameter that showed the largest statistically significant changes was MMO, with a significant difference ($p < 0.001$) between the groups after 6 and 12 days of treatment (Fig. 4). After 6 days of treatment, the 95% *CI*s were as follows: 37.707–38.492 (CG MMO) vs. 39.161–40.439 (KTG MMO); 6.28–6.7 (CG LLM) vs. 6.449–7.091 (KTG LLM); 6.148–6.592 (CG RLM) vs. 7.491–7.969 (KTG RLM). After 12 days of treatment, the 95% *CI*s were as follows: 39.227–39.973 (CG MMO) vs. 42.479–43.121 (KTG MMO); 7.061–7.519 (CG LLM) vs. 6.817–7.383 (KTG LLM); 6.888–7.392 (CG RLM) vs. 7.653–8.147 (KTG RLM).

Figure 5 shows the results of the NRS pain intensity analysis in the KTG and CG before treatment (1), after 6 days of treatment (2) and after 12 days of treatment (3).

A significant difference was observed between the groups in the analysis of the pain intensity parameter ($p < 0.001$). The patients who received KT in combination with TE experienced better pain relief than the CG (Fig. 5).

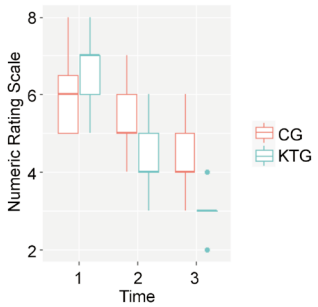

Fig. 5. Statistical analysis of the pain intensity in the KTG and CG before treatment (1), after 6 days of treatment (2) and after 12 days of treatment (3)

The 95% *CI*s after 6 days of therapy were 4.888–5.392 (CG NRS) vs. 3.973–4.627 (KTG NRS). The 95% *CI*s after 12 days of therapy were 4.164–4.576 (CG NRS) vs. 2.762–3.178 (KTG NRS).

Figure 6 shows the statistical analysis results for the PSS-10 (in stens and points) before treatment (1) and after 12 days of treatment (3) in the KTG and CG. The analysis of the PSS-10 results indicates that therapy using KT and TE significantly reduced perceived stress intensity compared to the CG ($p < 0.05$) (Fig. 6).

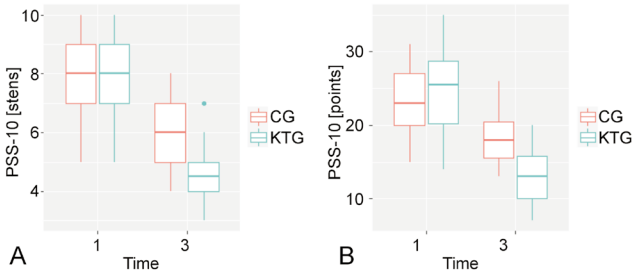

Fig. 6. Statistical analysis of the Perceived Stress Scale (PSS-10) in the KTG and CG expressed in stems (A) and points (B) before treatment (1) and after 12 days of treatment (3)

Ancillary analyses

Table 4 presents the results of the *MID* analysis. The analysis focuses on changes in the patient's reported results (beneficial or harmful) that are significant enough to justify a change in the patient's management.[43] Minimally important significant differences were found for the MMO, sEMG and PSS-10 parameters (Table 4).

Table 4. Results of minimal important difference (*MID*) analysis

Variable	*M*	*SD*	*MID*
MMO [mm]	36.2	1.49	0.746*
RLM [mm]	6.29	0.897	0.448
LLM [mm]	5.94	0.846	0.423
NRS	6.29	0.947	0.474
sEMG RLX [μV]	26.5	3.82	1.91*
sEMG MVC [μV]	286	81.6	40.8*
sEMG %MVC [%]	10.4	3.29	1.65*
PSS-10 [points]	23.8	5.17	2.58*
PSS-10 [stens]	7.72	1.45	0.726*

M – mean; * statistically significant.

Possible harms

No adverse effects were reported by any patient during the study. However, as KT tape is visible on the patient's face, it may cause mild social discomfort for sensitive individuals. Additionally, there have been some minor publications regarding hypersensitivity to KT glue. However, none of our patients reported any complaints.[44]

Discussion

The current study aimed to assess the therapeutic efficacy of KT and TE in female patients with pain, increased masticatory muscle tension and limited MMO. Our clinical observations have shown a significant increase in the number of patients with this profile. Similar observations have been reported by other authors.[45–47]

The study results indicate that both self-treatment and KT combined with TE had a significant effect on the change in MAS sEMG at rest and during exercise ($p < 0.05$). However, it is important to note that the measure of power (partial eta squared (pes); ANOVA) obtained in the study was poor (RLX: pes = 0.007; MVC: pes = 0.074; %MVC: pes = 0.017). There was no statistically significant difference between the effects of the 2 treatments on muscle bioelectric activity ($p > 0.05$). However, the *MID* analysis showed a significant difference in the sEMG parameter due to the applied therapies (RLX: *MID* = 1.91; MVC: *MID* = 40.8; %MVC: *MID* = 1.65). Nevertheless, the difference between the 1st measurement point (after 6 days of therapy) and the 3rd measurement point (after 12 days of treatment) was minimal in both groups. Similarly, Rocha Dutra et al. did not find a significant difference in sEMG between the KTG and the CG ($p = 0.1494$).[36] In a study by Soylu et al. that evaluated the short-term effect of masseteric KT on selected sEMG parameters related to fatigue and muscle strength during MVC, the effect of KT on sEMG parameters was not significant before or after KT application in healthy subjects.[48] In contrast, a study by Rathi et al. evaluating the effect of KT on MAS bioelectric activity and pain in patients with bruxism showed that KT markedly improved MAS activity and reduced pain. Additionally, a significant carry-over effect was observed after tape removal (the effect persisted for 24 h).[49] Therefore, dynamic patching may have an impact on the bioelectric function of the MAS in patients with TMD. However, due to the limited number of scientific reports, conflicting results and small study groups, any conclusions should be made with caution. Further studies using EMG are necessary to accurately determine the effect of KT on MAS bioelectrical function.

The assessment of the effect of the different therapies on improving TMJ ROM revealed a significant improvement in the KTG compared to the CG after 12 days, with a statistically significant difference in

MMO found in subjects who received dynamic patching ($p < 0.001$). Indeed, KT may contribute to improvements in TMJ function that result in increased mouth opening. When analyzing the *MID*, the applied therapies led to a clinically effective difference in the MMO parameter (MMO = 0.746), with the KTG showing improvement compared to the CG. After 6 days of therapy, the KTG demonstrated a more substantial difference in mouth opening, and after 12 days, the KTG had a more than twofold improvement in this parameter. In contrast, no *MID* was demonstrated in the LLM and RLL parameters (LLM: *MID* = 0.423; RLL: *MID* = 0.448). Therefore, the use of KT therapy may prove to be an effective adjunctive method for patients with TMD and limited mouth opening.

A statistically significant difference in pain intensity level on the NRS was observed between the KTG and CG ($p < 0.001$). The application of KT to the MAS showed an analgesic effect in patients with TMD, leading to an improvement in mouth opening. This therapeutic effect was already achieved after 6 days of therapy. Benlidayi et al. found that adding KT was more effective than counseling and training alone in improving several factors in 28 TMD patients, including pain reduction ($p = 0.001$), improvement in ROM ($p = 0.003$), disability ($p = 0.010$), and psychological status ($p = 0.000$).[40] Therefore, the increased TMJ ROM may be related to the pain-relieving effects of KT, which may have facilitated the implementation of TE in subjects.

Tran et al. conducted a meta-analysis of 36 research papers comparing the effectiveness of KT with other methods for the treatment of musculoskeletal disorders.[50] The results showed that KT improved pain and disability in all body areas. Within the first 5 days of use, KT significantly reduced pain in all body regions (SMD = –0.63, 95% *CI*: –0.87––0.39).[50] Furthermore, after 4–6 weeks of use, KT improved disability in all body areas (SMD = –0.59, 95% *CI*: –0.96––0.22).[50] Uzma's study compared the effectiveness of KT and conventional therapy with myofascial and traditional treatment in TMD patients. A within-group comparison showed improvement in both groups after 1 week. However, the experimental group demonstrated a significant improvement ($p = 0.05$). Therefore, KT is beneficial for reducing pain or improving ROM in TMD patients.[51]

Volkan-Yazici et al. compared the effects of manual therapy (MT) alone with MT combined with KT in patients with bruxism. The results showed that both methods were effective in treating bruxism, with the combination of MT and KT resulting in further reduction of jaw pain and temporal pain compared to MT alone.[52] In a meta-analysis by Meneses Emérito et al., taping was found to provide significant pain relief (measured using the visual analog scale (VAS)) after 1 week of treatment, compared to other methods analyzed. However, the authors noted that the limited number of studies and their biases limited the results.[53]

Baklaci reached a significant conclusion when comparing the effectiveness of treating TMD through relaxation splinting and KT.[54] Both the KT and splint groups experienced a reduction in pain ($p < 0.01$) and a significant increase in ROM ($p < 0.05$). However, there were no significant differences in VAS and ROM between the 2 groups. Moreover, both groups demonstrated improvements in daily eating activity and sleep quality, although no such improvements were observed in other oral activities.[54] Keskinruzgar et al. compared the therapeutic efficacy of KT and chiropractic therapy in patients with sleep bruxism and found a statistically significant difference between the KT and splint groups in terms of masseter and temporal muscle pressure pain thresholds (MPPT, TPPT), VAS, and mouth opening values before treatment and at weeks 1 and 5, except for TPPT values at week 1, which were higher in the kinesiology group than the splint group ($p < 0.05$).[55] Thus, the study suggests that KT is at least as effective as an occlusal splint in the treatment of sleep bruxism.[55]

In contrast, a literature review of 34 articles by Cheshmi et al. concluded that KT is not a reliable standalone treatment option for craniomaxillofacial disorders. However, it is considered a useful complementary option to improve treatment outcomes in a variety of conditions.[56] The study found a reduction in perceived stress in the KT group compared to the CG ($p < 0.001$). Analysis of the *MID* showed that the therapies used had a clinical effect on the PSS-10 scores and sten scores (*MID* = 2.58 and *MID* = 0.726, respectively). Patients receiving KT and TE exhibited a greater reduction in the PSS-10 score compared to the CG.

He et al. studied the efficacy of KT in patients with TMD by assessing its impact on various parameters, including the Self-Rating Anxiety Scale (SAS) and the Self-Rating Depression Scale (SDS), over a 6-day period. The results demonstrated that KT effectively improved the mood of TMD patients.[57] Our results, along with those of other authors, suggest that KT has a beneficial effect on stress parameters, which is particularly important in the treatment of TMD patients, as the literature reports that patients with these disorders often have high levels of stress, anxiety and depression.[58]

The results of the current study indicate that KT, in combination with TE, is an effective tool for reducing pain and improving mandibular ROM in female TMD patients. The authors of this paper and other researchers suggest that the effect of KT on the psychoemotional state may play a significant role in this process.[40] In addition, the potential risks or complications associated with the use of KT appear to be very low. It is a non-invasive method that is easy to use and can be removed if necessary. However, due to the paucity of scientific studies, the heterogeneity of methods and the small treatment groups, it is recommended that the therapeutic effect described be approached with caution. Arguably, in everyday clinical practice, KT may prove to be an effective adjunct to well-established TMD treatments. Based on current

knowledge, KT should be considered a tool to sustain the therapeutic effect of standardized and well-described therapies. Further studies are necessary to evaluate the effectiveness of KT in treating TMD and to determine its therapeutic potential tangibly and unequivocally.

Limitations

The present study initially aimed to assess the therapeutic effects of KT in female patients with pain, increased MAS tension and limited TMJ mobility. There were several limitations to this study. First, the sample size was relatively small, which limited the generalizability of the results. Second, the study had a short intervention period (12 days), which will be extended in future studies to assess whether the evaluated indicators change over the course of treatment, providing a better understanding of the effects of the KT intervention. Additionally, the conducted studies lacked a comparative placebo group. Finally, the duration of treatment effects was not analyzed after the end of treatment. The authors will continue their study on the effectiveness of KT in treating TMD, with a focus on the abovementioned limitations.

Conclusions

The combination of KT, counseling and MAS exercises provides additional therapeutic benefits by increasing TMJ mobility and reducing pain severity compared to exercise alone. Therefore, KT could be an effective form of complementary therapy in TMD management. Additionally, KT combined with TE demonstrated a beneficial effect on perceived stress levels, which is a novel finding. However, further insight and additional studies are required to fully understand this phenomenon, including psychological assessment of TMD patients. Clinicians, physiotherapists and orofacial pain practitioners should consider implementing KT and TE in patients with painful TMD who have elevated pain sensitivity. Patients who catastrophize or have severe symptoms that could be intensified by more intense PT treatment, such as MT, may benefit from alternative treatment modalities.

Ethics approval and consent to participate

The study was approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin (approval No. KB - 0012/102/13). Informed consent was obtained from all study participants.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Magdalena Gębska https://orcid.org/0000-0002-1477-8302
Bartosz Dalewski https://orcid.org/0000-0002-0652-664X
Łukasz Pałka https://orcid.org/0000-0002-9365-7882
Paweł Kiczmer https://orcid.org/0000-0001-9060-0154
Łukasz Kołodziej https://orcid.org/0000-0002-0400-7918

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Original paper

Color perception and its relation to dental anxiety in children

Prabhadevi Chandrashekara Maganur[1,B], Satish Vishwanathaiah[1,C], Mir Faeq Ali Quadri[2,C], Manal Alsabi[3,C,D], Wafaa Modarba[3,C], Wejdan Aqeel[3,C], Natheer Al Rawi[4,C], Asmaa Uthman[5,C,E], Rodolfo Reda[6,A], Mohammad Jafer[1,D], Luca Testarelli[6,E], Shankargouda Patil[7,8,E,F]

[1] Department of Preventive Dental Sciences, College of Dentistry, Jazan University, Saudi Arabia
[2] Woody L. Hunt School of Dental Medicine, Texas Tech University Health Sciences Center El Paso, USA
[3] College of Dentistry, Jazan University, Saudi Arabia
[4] College of Dental Medicine, University of Sharjah, United Arab Emirates
[5] Department of Diagnostic and Surgical Dental Sciences, College of Dentistry, Gulf Medical University, Ajman, United Arab Emirates
[6] Department of Oral and Maxillofacial Sciences, Sapienza University of Rome, Italy
[7] College of Dental Medicine, Roseman University of Health Sciences, South Jordan, USA
[8] College of Graduate Studies, Roseman University of Health Sciences, South Jordan, USA

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):671–677

Address for correspondence
Rodolfo Reda
E-mail: rodolforeda17@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on August 1, 2021
Reviewed on January 1, 2022
Accepted on January 16, 2022

Published online on October 25, 2024

Cite as
Maganur PC, Vishwanathaiah S, Ali Quadri MF, et al. Color perception and its relation to dental anxiety in children. *Dent Med Probl.* 2024;61(5):671–677. doi:10.17219/dmp/145896

DOI
10.17219/dmp/145896

Abstract

Background. One of the major causes of dental anxiety in children is their first impression of the dental environment. Even minor details, such as the choice of color in a dental setting and the color of dental equipment, can positively influence a child's behavior.

Objectives. The aim of the study was to assess the relationship between the emotions in children and color combinations in a pediatric setting.

Material and methods. The study involved 200 children (99 boys and 101 girls) aged between 6 and 12 years who visited the dental clinics at the College of Dentistry, Jazan University, for the first time between November 2017 and January 2018. The participants were divided into 2 groups based on age. The younger children group included participants aged from 6 to 9 years, while the older children group included participants aged from 10 to 12 years. Anxiety levels were recorded using the Modified Dental Anxiety Scale. Colored pencils and images of emoticons were provided to all children, who were instructed to color the negative and positive emoticons with their preferred colors.

Results. The analysis of anxiety levels among children in both groups revealed statistically significant differences across sexes in the younger age group, with girls being more anxious than boys ($p = 0.003$). Additionally, a statistically significant difference was observed in the choice of colors by children of both sexes in 2 age groups ($p = 0.001$). Most children were inclined towards bright colors and used them to express their emotions.

Conclusions. The incorporation of colors in a dental setting could invoke positive emotions in children. Hence, the use of colors in the workplace has the potential to ease anxiety.

Keywords: emotions, anxiety, dental anxiety, anxiety scale, colors

Introduction

From the moment of their birth, humans have been subject to fear and emotions. Four causes of fear have been identified: non-associative perspective or direct stimulation; physiological differences; an innate predisposition; and conditioning.[1] For a long time, concerns have been raised regarding the reasoning behind dental anxiety in children. It remains a major obstacle in the field of dental care. Dental anxiety in children is attributed to a multitude of factors. Children may become anxious directly or through indirect learning or conditioning (from information or by modeling).[2] The overall comprehension of the dental environment is a notable cause of anxiety in children.[3] Shifting the perspective of society and parents could motivate pediatric dentists to develop a child-oriented environment to help children, especially when uncooperative and anxious.[4] Environmental aspects such as colors leave a positive imprint in the minds of children, which in turn reduces dental anxiety to a considerable extent. Thus, colors play a vital role in a child's life.[5,6]

For children, color preference is expressed through their belongings and surroundings, such as toys, clothing, lunch boxes, sportswear, and home accessories.[7] Research has demonstrated the relationship between the performance and behavior of a child and their color preferences.[6,8] Colors have been classified as warm or cool, based on their correlation with temperature.[9] Colors have 3 fundamental features, namely saturation, value and hue. The human eye is able to recognize colors through cones and rods. After the color passes into the eye via the retina, receptor cells absorb the hues, and the corresponding signal is sent to the brain for deciphering. Simultaneously, the hormone-regulating endocrine glands receive these brain impulses. Hence, color represents both psychological and physiological perceptions, triggering emotional responses.[10]

The results of numerous studies have demonstrated that children use colors as a medium to express their emotions. Moreover, the coordination between the brain, eyes and light creates a standard reaction to color.[5,11] Therefore, pediatric dentistry can utilize child-friendly colors to positively influence children's emotional status.[12] It has been observed that the choice of colors in a dental office and treatment room has a positive effect in addressing dental anxiety. The use of bright colors and colored equipment helps children maintain a calm composure during their dental visits.[3]

The present study aimed to assess 2 assumptions. The first assumption states that a significant association exists between color preference and emotions in children. The second assumption posits that there is a sex-based discrepancy in the level of anxiety among children. Accordingly, it is hypothesized that there is a correlation between the use of bright colors in a dental clinic and the level of dental anxiety among children. Also, there is a possibility of variation in the level of anxiety between males and females. Therefore, the present study focused on examining the relationship between children's age, sex and levels of anxiety before dental procedures, as well as testing the psychometric properties of the dental anxiety scale and identifying any differences in children's color preferences by age and sex when asked to color a happy or a sad emoticon.

Material and methods

Study setting and participants

The study was conducted at the Pediatric Dental Clinic of the College of Dentistry (Jazan University, Saudi Arabia), and approved by the Ethics Committee of Jazan University (approval No. CODJU-1716I). The study participants were children between the ages of 6 and 12 years who were visiting the Pediatric Dental Clinic for the first time. Individuals who were not first-time visitors, undergoing psychiatric therapy, or suffering from generalized anxiety disorders were excluded from the study. The purpose and procedure of the study were explained to the children and their accompanying parents or guardians. Written consent was obtained from the parents or legal guardians of the study participants before the start of the study.

Sample size estimation

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

$$N = (4pq)/d^2 \quad (1)$$

where:
N – sample size;
p – proportion of children with anxiety (66.7%);
q – 1–p;
d – precision of the study [%].

The sample size required for the present study was calculated to be 181,[13] with a precision of 7%.

Study design

This cross-sectional descriptive survey was conducted from November 2017 to January 2018. The children who participated in the study were divided into 2 groups based on age. The younger group consisted of children between the ages of 6 and 9, while the older group ranged between the ages of 10 and 12. The personal details of each child were recorded before the study and were kept confidential. An Arabic translation of the Modified Dental Anxiety Scale, which includes emoticons (sad and happy), was carried out.[14] A validation test for the dental anxiety scale

based on the study by Humphris et al. was conducted before the study.[15] While the dependent variable was the level of anxiety among children on account of dental procedures, the independent variables included age, sex and color preferences on the level of anxiety.

Arabic validation of the anxiety scale

A dental intern fluent in Arabic had initially translated the questions that form part of the Modified Dental Anxiety Scale from English to Arabic (available on request from the corresponding author).[16] This was followed by a reverse translation conducted by a bilingual dentist. Minor errors were corrected through mutual coordination. Additionally, a biostatician was consulted to validate the questions and assess the intended outcome (single global construct). Experts were responsible for rationally analyzing the questionnaire by focusing on clarity, comprehensiveness, readability, and the level of agreement using the Likert Scale. To assess the reliability of the Arabic version of the Modified Dental Anxiety Scale, test–retest reliability and internal consistency were calculated using Cronbach's alpha coefficient. It was stated that the Arabic version would be internally consistent if the alpha coefficient had a minimum value of 0.70.[16] Furthermore, if the value obtained for the intraclass correlation (ICC) agreement calculated using Pearson's r was 0.80 or more, the questionnaire would be considered reliable (excellent).[17] Confirmatory factor analysis corroborated the validity of the child anxiety questionnaire. It was established that each item in the model must contribute to and correlate with the single global construct.[18,19]

Analysis of variables

The present study evaluated 3 variables: demographics; the dental anxiety scale; and the choice of color for emoticons. A single examiner was responsible for recording the readings in order to ensure uniformity. The Arabic version of the Modified Dental Anxiety Scale employed in the present study consisted of a series of 5 questions presented to the participants for the purpose of rating their level of anxiety in relation to a specific dental situation according to their own perception. The questionnaire was completed by the participants during their visit to the dental clinic, with the assistance of their parents or guardians when necessary. The responses were evaluated on a five-point Likert Scale ranging from 1 (no anxiety) to 5 (extreme anxiety). Therefore, the total scores ranged from 5 to 25. A score below 15 was indicative of a lack of anxiety, while a score of 15 or above was indicative of anxiety. This classification was adapted from the study by Annamary et al.[16] To identify any potential associations between color and pain perception, a set of 8 colored pencils (black, white, pink, orange, green, blue, yellow, and red) was given to each child. The participants were required to color 2 emoticons, representing happiness and sadness, with the color of their choice from the provided set. The recorded data was then tabulated and analyzed.

Statistical analysis

The IBM SPSS Statistics for Windows software, v. 24.0 (IBM Corp., Armonk, USA), was used for the statistical analysis. The data was first entered into an Excel spreadsheet and subsequently transferred to the IBM SPSS Statistics for Windows software dataset. The relationships between the dependent and independent variables were analyzed using the χ^2 test. The significance level was set at $p < 0.05$.

Results

The results of the validation test demonstrated that there were no notable discrepancies in the test–retest reliability. An ICC value of 0.81 was obtained using Pearson's r, which demonstrated the questionnaire's excellent reliability (Table 1). The results of the factor analysis are shown in Table 2. The 5 items included in the questionnaire made a significant contribution to the single global construct, thereby increasing the credibility of the tool in assessing anxiety levels among children.

A total of 200 children were included in the study. Of the participants, 99 (49.5%) were male and 101 (50.5%) were female. The younger group consisted of 79 children, 35 of whom were male and 44 of whom were female. The older group comprised 121 children (64 boys and 57 girls). Overall, based on the Modified Dental Anxiety Scale, 35 children were anxious and 165 children were non-anxious. The study revealed statistically significant differences between the boys and girls in the younger age group, with a p-value of 0.003. However, no statistically significant difference was observed in the older age group. In both age groups, girls exhibited greater levels of anxiety than boys (Table 3).

Table 1. Test–retest reliability of the Arabic version of the Modified Dental Anxiety Scale

Instrument	ICC	Cronbach's alpha
Arabic version of the Modified Dental Anxiety Scale	0.81 ±0.08	0.75

ICC – intraclass correlation.

Table 2. Confirmatory factor analysis of the Arabic version of the Modified Dental Anxiety Scale

Item	Item 1	Item 2	Item 3	Item 4	Item 5
Item 1	1.00	–	–	–	–
Item 2	0.89	1.00	–	–	–
Item 3	0.86	0.91	1.00	–	–
Item 4	0.90	0.83	0.85	1.00	–
Item 5	0.91	0.83	0.90	0.89	1.00

Table 4 shows the results of the comparative analysis conducted to assess the relationship between color preferences related to anxiety levels and positive emotions. The results revealed no statistically significant differences in color preferences between boys and girls in both age groups. Blue, pink and yellow colors were preferred by both anxious and non-anxious younger children. In the older group, children exhibiting anxiety symptoms preferred pink to other colors, while the non-anxious participants preferred blue, yellow and pink over other colors. None of the children chose the color black to express their emotions.

A similar comparative analysis was conducted to assess color preferences in relation to anxiety levels and negative emotions (Table 5). The findings show that the anxious children belonging to the younger age group preferred black and red to express negative emotions, while the non-anxious children belonging to the same age group preferred red, black and yellow over other colors. Among the older participants, those exhibiting anxiety symptoms preferred black, whereas the non-anxious children preferred red, black and yellow over other colors.

Our study also analyzed the association of colors with both positive and negative emotions among children of both sexes across 2 age groups. In the older children group, boys preferred blue and yellow when expressing positive emotions, while girls exhibited a preference for pink and yellow. In the younger group, however, the male participants chose yellow, blue and green, whereas the female participants selected pink, red and blue to express positive emotions. Both boys and girls demonstrated similarities in ignoring the color white. A statistically significant difference in the choice of colors by children of both sexes was observed in 2 age groups, with a *p*-value of 0.001 (Table 6), thereby providing evidence to reject the null hypothesis and accept the alternative hypothesis. Consequently, it can be concluded that the use of bright colors decreases the level of anxiety among children.

In expressing negative emotions, children in the older age category preferred black and red over other colors, with white being among the least preferred colors. Among the younger children, while the majority of boys expressed their emotions through black and red, girls preferred black, red and yellow (Table 7). Therefore, it can be inferred that the use of bright colors has a positive impact on reducing dental anxiety in children.

Table 3. Anxiety levels among boys and girls in younger and older age groups

Age group	Sex	Level of anxiety		Total	*p*-value
		anxious	non-anxious		
Younger children	male	6 (17.1)	29 (82.9)	35 (44.3)	0.003*
	female	13 (29.5)	31 (70.5)	44 (55.7)	
Older children	male	6 (9.4)	58 (90.6)	64 (52.9)	0.180
	female	10 (17.5)	47 (82.5)	57 (47.1)	

* statistically significant ($p < 0.05$, χ^2 test, degrees of freedom (df) = 1). Data presented as frequency (percentage) (n (%)).

Table 4. Comparative analysis of color preferences in relation to the level of anxiety and positive emotions across 2 age groups

Age group	Level of anxiety	Positive emotions (happy face)							*p*-value
		red	blue	green	pink	white	yellow	black	
Younger children	anxious (n = 19)	3 (15.8)	4 (21.1)	3 (15.8)	4 (21.1)	1 (5.3)	4 (21.1)	0 (0.0)	0.370
	non-anxious (n = 60)	10 (16.7)	12 (20.0)	10 (16.7)	14 (23.3)	3 (5.0)	11 (18.3)	0 (0.0)	
Older children	anxious (n = 16)	3 (18.8)	3 (18.8)	2 (12.5)	5 (31.3)	1 (6.3)	2 (12.5)	0 (0.0)	0.510
	non-anxious (n = 105)	10 (9.52)	34 (32.4)	9 (8.6)	23 (21.9)	2 (1.9)	27 (25.7)	0 (0.0)	

* statistically significant ($p < 0.05$, χ^2 test, df = 6). Data presented as n (%).

Table 5. Comparative analysis of color preferences in relation to the level of anxiety and negative emotions across 2 age groups

Age group	Level of anxiety	Positive emotions (happy face)							*p*-value
		red	blue	green	pink	white	yellow	black	
Younger children	anxious (n = 19)	5 (26.3)	7 (36.8)	0 (0.0)	4 (21.1)	1 (5.3)	0 (0.0)	2 (10.5)	0.370
	non-anxious (n = 60)	15 (25.0)	15 (25.0)	4 (6.7)	5 (8.3)	3 (5.0)	3 (5.0)	15 (25.0)	
Older children	anxious (n = 16)	2 (12.5)	7 (43.8)	1 (6.3)	1 (6.3)	1 (6.3)	3 (18.8)	1 (6.3)	0.130
	non-anxious (n = 105)	29 (27.6)	19 (18.1)	9 (8.6)	14 (13.3)	8 (7.6)	7 (6.7)	19 (18.1)	

* statistically significant ($p < 0.05$, χ^2 test, df = 6). Data presented as n (%).

Table 6. Comparative analysis of color preferences associated with positive emotions among boys and girls in 2 age groups

Age group	Sex	Positive emotions (happy face)							*p*-value
		red	blue	green	pink	white	yellow	black	
Younger children	male (*n* = 35)	4 (11.4)	9 (25.7)	9 (25.7)	0 (0.0)	2 (5.7)	11 (31.4)	0 (0.0)	0.001*
	female (*n* = 44)	9 (20.5)	7 (15.9)	4 (9.1)	18 (40.9)	2 (4.5)	4 (9.1)	0 (0.0)	
Older children	male (*n* = 64)	10 (15.6)	28 (43.8)	7 (10.9)	1 (1.6)	1 (1.6)	17 (26.6)	0 (0.0)	0.001*
	female (*n* = 57)	3 (5.3)	9 (15.8)	4 (7.0)	27 (47.4)	2 (3.5)	12 (21.1)	0 (0.0)	

* statistically significant ($p < 0.05$, χ^2 test, $df = 6$). Data presented as *n* (%).

Table 7. Comparative analysis of color preferences associated with negative emotions among boys and girls in 2 age groups

Age group	Sex	Positive emotions (happy face)							*p*-value
		red	blue	green	pink	white	yellow	black	
Younger children	male (*n* = 35)	13 (37.1)	8 (22.9)	2 (5.7)	2 (5.7)	2 (5.7)	3 (8.6)	5 (14.3)	0.030*
	female (*n* = 44)	7 (15.9)	14 (31.8)	2 (4.5)	7 (15.9)	2 (4.5)	0 (0.0)	12 (27.3)	
Older children	male (*n* = 64)	17 (26.6)	11 (17.2)	6 (9.4)	7 (10.9)	9 (14.1)	7 (10.9)	7 (10.9)	0.080
	female (*n* = 57)	14 (24.6)	15 (26.3)	4 (7.0)	8 (14.0)	0 (0.0)	3 (5.3)	13 (22.8)	

* statistically significant ($p < 0.05$, χ^2 test, $df = 6$). Data presented as *n* (%).

Discussion

From the moment of birth, man is subject to anxiety and fear. These emotions have a multifactorial and complex physiological and psychological etiology in dental environments. Dental anxiety represents the most significant challenge and a major concern for pediatric dentists. Many dental procedures are either avoided or missed intentionally when children are anxious, largely due to the erroneous belief that the procedure will be excruciatingly painful. The avoidance of dental visits has a detrimental impact on children's oral health. The present study revealed that the dental environment plays an important role in reducing anxiety levels in children. Umamaheshwari et al. observed that the primary cause of dental anxiety is a negative perception of the dental environment by children.[3] Pediatric dentists must, therefore, create a calm and welcoming atmosphere in their clinics to address the concerns of young patients.

In the present study, non-anxious children from both groups preferred blue, pink and yellow when coloring a happy face. Younger anxious participants, however, preferred yellow, blue and pink, whereas older anxious children preferred pink, red and blue over other colors. This observation is in accordance with the study by Umamaheshwari et al., who observed that younger children associate yellow with positive emotions, while older children prefer blue to depict positive emotions.[3] Furthermore, the present study noted that younger anxious children preferred black and red to express negative emotions, while older children preferred black to express the same emotions. In the case of non-anxious participants, both groups selected red, black and yellow to represent negative emotions. In contrast with the results of the present study, Odom and Sholtz evaluated the impact of hue on mood tones. The findings indicated that blue had a calming effect, while yellow was perceived as both exciting and cheerful. While younger anxious children preferred black, older children preferred red for expressing negative emotions.[20]

The current study also noted that younger children with anxiety preferred the color black to depict negative emotions, while the older group preferred red and black. This finding aligns with the results of the study conducted by Boyatzis and Varghese, which observed a correlation between darker colors and negative emotions as well as lighter colors and positive emotions.[6] The hypothalamus controls the nerve centers, respiration and heart rate. It induces a physical reaction in children when they are exposed to light stimuli and different colors. Every energy and wavelength has a different impact on children. Specific colors have been observed to increase perspiration, cause a vascular reflex action, stimulate muscular reactions, and affect the eye blinking rate. Kurt and Osueke observed that both violet and blue can lower blood pressure, while yellow, red and orange evoke different responses.[21] With regard to the relationship between color temperature and children's reactions, it was observed that while cool or warm colors could calm one child, they may evoke a different response in another child.[20]

The *p*-value of 0.003 observed in the current study rejects the null hypothesis and accepts the alternative hypothesis, concluding that there was a sex variation in the anxiety levels among younger children. The present study indicates that the majority of boys in both groups preferred red and black to express negative emotions. With regard to positive emotions, younger girls chose pink, blue and red, while the older ones selected pink, blue and yellow. Pink was the leading color choice among girls. Both younger and older girls, 31.8% and 26.3%, respectively, picked black to express negative emotions. In both age groups, the boys selected yellow and blue to depict positive emotions. These results bear a resemblance to a previous study by Annamary et al.[16] Some experts believe that the cultural influences account for these preferences. Typically, parents raise boys in a blue environment and girls in a pink environment.[5]

A study conducted by Jayakaran et al. in 50 randomly selected children aged 6–10 years aimed to determine the preferred aids that help them cope with their anxiety levels.[7] The authors observed that the most preferred aids were watching cartoons, listening to music, observing cartoon-painted walls, playing with toys, and the presence of parents. Implementing these aids and designing the pediatric clinics accordingly will ensure a child-friendly environment and thereby improve the quality of healthcare.[7]

In a study by Hotwani and Sharma, 100 children aged 9 years were assessed for their preference of local anesthesia and the impact of colors on their anxiety levels using the faces version of the Modified Child Dental Anxiety Scale.[22] In the study, the child was asked to match the emotions with the 6 colored injectors according to their preference. It was observed that a change in physical appearance and color helped to reduce anxiety levels, and thus could be considered a modality of behavior management. Goldstein had previously observed that specific colors can elicit an emotional response.[23] Other researchers have also stated that the choice of color used in children's artwork reflects their underlying emotional state.[23–25]

Based on the environment, parents can indirectly influence their children's color preferences. Furthermore, experiences and nationality can also influence color preferences.[23] The relationship between colors and their combination and emotions has been investigated in several studies.[26] The use of colors in a dental setting can contribute to a sense of calm for both staff and children. The use of colored equipment can further expand this benefit. It was agreed that 7 colors should be used in the study (black, red, white, yellow, green, blue, and pink). Blue, green, yellow, and red were chosen because they represent the 4 basic colors of the Munsell color system. Pink was selected as the color of bodily tissues, and black and white were selected because they are achromatic colors.[26] The emoticon set included sad and happy faces. Terwogt and Hoeksma found that the association between emotions and color preferences differed between age groups.[27] While children in the older age group displayed a lower correlation between emotions and colors, children in the younger age group demonstrated a stronger correlation.[27] This aligns with the finding that, depending upon personal experiences, as a person grows older, their choices and preferences vary.[27]

The dental environment plays a crucial role in children's behavior, particularly in their willingness to cooperate with treatment. The emotional state of the child is reflected in their physical health.[28] In their study on the use of color in pediatric dentistry, Park and Park showed that the use of child-friendly colors can positively impact emotional health.[12] Their findings indicated that girls exhibited greater preference for red and purple than boys. It was also observed that healthy children and pediatric patients preferred blue and green, with a lesser preference for white. The color red is associated with pain, while yellow is associated with the absence of pain, as observed among Turkish children by Altan et al.[29] Another longitudinal cohort study revealed an increased prevalence of dental fear and anxiety (DFA) among children between the ages of 7 and 9.[2] The development of new carious lesions, the experience of toothache and extractions were identified as the most significant risk factors for the development of DFA. According to the study, dental treatment should not only focus on dental care but also consider its psychological impact, thereby preventing painful and traumatic experiences.[2]

A reduction in anxiety levels would decrease the time required for dental procedures and would make the procedure less stressful for the patient. Therefore, the present study suggests that by determining the levels of anxiety and identifying child-friendly colors, it is possible to incorporate these colors into the healthcare environment. This could be helpful in reducing anxiety in children and facilitating dental procedures with better success rates. Furthermore, a dental environment free of anxiety would facilitate the expression of dental concerns by children.

Limitations

The present study evaluated only 2 emotions, happiness and sadness. Further prospective and longitudinal studies are required to evaluate a greater variety of emotions in a larger population.

Conclusions

The impact of color on feelings and emotions is contingent upon the color perspective, the overall environment and the type of emotional link experienced by the individuals. Based on the results of our study, it can be presumed that there is a significant correlation between color perceptions and emotions. These factors have a major impact on children, depending on their anxiety

level, sex and age. Hence, the implementation of a child-friendly environment in dental clinics would reduce anxiety levels and facilitate cooperation with pediatric patients.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Jazan University (approval No. CODJU-1716I). Written consent was obtained from the parents or legal guardians of the study participants before the start of the study.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Prabhadevi Chandrashekara Maganur https://orcid.org/0000-0002-0959-2597
Satish Vishwanathaiah https://orcid.org/0000-0002-8376-297X
Mir Faeq Ali Quadri https://orcid.org/0000-0003-2988-6602
Manal Alsabi https://orcid.org/0000-0002-2189-3210
Wafaa Modarba https://orcid.org/0000-0001-7625-9674
Wejdan Aqeel https://orcid.org/0000-0001-5988-8735
Natheer Al Rawi https://orcid.org/0000-0002-7483-6594
Asmaa Uthman https://orcid.org/0000-0002-9362-4204
Rodolfo Reda https://orcid.org/0000-0003-1532-6524
Mohammad Jafer https://orcid.org/0000-0003-2263-7187
Luca Testarelli https://orcid.org/0000-0003-3904-3000
Shankargouda Patil https://orcid.org/0000-0003-0783-8263

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Original paper

Association between Behçet's disease and apical periodontitis: A cross-sectional study

Meltem Sümbüllü[1,C,D,F], Ayhan Kul[2,A,B], Ertuğrul Karataş[1,A,C,E], Meltem Memiş[3,B]

[1] Department of Endodontics, Faculty of Dentistry, Atatürk University, Erzurum, Turkey
[2] Department of Physical Medicine and Rehabilitation, Atatürk University, Erzurum, Turkey
[3] Rize Oral and Dental Health Center, Turkey

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):679–685

Address for correspondence
Meltem Sümbüllü
E-mail: meltem_endo@hotmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on November 9, 2022
Reviewed on December 18, 2022
Accepted on April 7, 2023

Published online on October 3, 2024

Abstract

Background. The correlation between Behçet's disease (BD) and apical periodontitis (AP) has not been investigated.

Objectives. The aim of the study was to evaluate the association between BD and AP using different variables.

Material and methods. A total of 98 individuals (49 with BD and 49 controls) were recruited for the study. The presence of AP was confirmed through radiographic and clinical examination in all patients. The following data was evaluated in both the BD group and the control group: the presence of teeth with AP; the presence of root canal-treated (RCT) teeth; the presence of RCT teeth with AP; the severity of the disease; the types of medication taken; and the duration of the disease. The χ^2 test and the logistic regression analysis were performed to ascertain the association between BD and AP.

Results. A total of 32 patients in the BD group and 12 patients in the control group presented with AP. The prevalence of teeth with AP was significantly higher in the BD group than in the control group (odds ratio = 5.804, $p < 0.05$). The χ^2 analysis demonstrated a statistically significant association between AP and both gender and BD activity ($p < 0.05$). Furthermore, the logistic regression analysis indicated that the severity of the disease was a predictor of BD ($p < 0.05$).

Conclusions. A significantly higher prevalence of AP was observed in patients with BD. However, the success rate of endodontic treatment in patients with BD was comparable to that observed in healthy individuals.

Keywords: Behçet's disease, Behçet's syndrome, apical periodontitis

Cite as
Sümbüllü M, Kul A, Karataş E, Memiş M. Association between Behçet's disease and apical periodontitis: A cross-sectional study. *Dent Med Probl.* 2024;61(5):679–685. doi:10.17219/dmp/163127

DOI
10.17219/dmp/163127

Introduction

Behçet's disease (BD), also known as Behçet's syndrome, is a form of multisystemic chronic vasculitis first defined by Dr. Hulusi Behçet in 1937. The disease affects the cardiovascular, nervous and gastrointestinal systems and presents with mucocutaneous lesions. The condition is a triple-symptom complex involving the oral, genital and ocular structures.[1] Behçet's disease affects both female and male individuals across a wide geographic area, but it is more prevalent among people along the so-called Silk Road.[2] The causes of BD include immune system disorder, genetic predisposition and endothelial cell dysfunction.[3] Since diagnostic tests cannot be performed for BD, the diagnosis is made based on clinical findings.[4]

Although the pathogenesis of BD is not fully understood, studies have demonstrated that immunological dysregulation plays an important role in its etiology and progression. An important part of the immunopathogenesis of BD is a T cell-mediated immune response.[5] T cells are divided into 2 groups, known as T helper cells 1 (Th1) and T helper cells 2 (Th2), which have antigen-specific receptors on their cell membranes for the identification of pathogens.[6] One of the key pathological immune characteristics of BD is the presence of elevated levels of pro-inflammatory Th1 cytokines (interleukin-2 (IL-2), IL-12, IL-18, IL-27 and interferon-γ (IFN-γ)) and Th2 cytokines (IL-2, IL-10, IL13, and tumor necrosis factor-β (TNF-β)), which play crucial roles in the disease.[7,8] It has been demonstrated that IL-6 is the main cytokine that functions as a promoter in patients with BD.[9,10] Furthermore, IL-6 and TNF provide both local and systemic responses to pathological stimuli.[11]

In the event of pulp necrosis, bacteria and their by-products reach the periradicular tissues via the apical foramen and lateral canals, thereby triggering inflammatory and immunologic reactions and causing apical periodontitis (AP).[12] T cells are one of the key factors involved in AP.[13] They produce cytokines, which may stimulate bone destruction and exert a pro-inflammatory function in AP.[13] The progression of AP and the subsequent bone resorption have been attributed to a response in Th1.[8]

Arabaci et al. indicated that periodontal status is influenced by the presence and severity of BD.[14] Another study demonstrated a correlation between periodontitis and BD activity.[15] Since AP and BD involve similar destructive and inflammatory reactions, there is a possibility of an association between the two. Thus, this cross-sectional study aimed to evaluate the correlation between BD and AP. The null hypothesis was that no association would be observed.

Material and methods

This study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement. The clinical study was conducted in the Department of Rheumatology, School of Medicine, and the Department of Endodontics, Faculty of Dentistry (Atatürk University, Erzurum, Turkey). The study was approved by the Ethics Committee of the Atatürk University (decision No. 71-09/2022). The sample size was calculated based on the data from a previous study,[16] with an effect size of 0.35, an alpha error of 0.05 and a power of 0.9. According to this analysis, the study should have included 85 patients.

The study population consisted of 49 patients with BD, aged 28–65 years, who were accepted for treatment at the endodontics clinic. The patients did not present with any additional systemic diseases apart from BD. The participants of the study met the criteria set forth by the International Study Group for BD.[17] The control group consisted of 49 sex- and age-matched patients who did not have any systemic diseases.

The BD activity index was used to determine the severity of the disease, with patients classified accordingly.[18] Each symptom was assigned a specific score. The overall disease activity was determined by summing the scores. Oral aphthous ulcers, arthralgia, genital ulcers, erythema nodosum, folliculitis, and papulopustular lesions were classified as mild symptoms and were assigned 1 point each. Moderate symptoms included arthritis, deep vein thrombosis of the legs, anterior uveitis, and gastrointestinal involvement. They were assigned 2 points each. Posterior uveitis/panuveitis, retinal vasculitis, arterial thrombosis, neuro-Behçet's disease, and bowel perforations were classified as severe symptoms and were assigned 3 points each.

Additionally, the duration of the disease and the types of medication administered were documented. The types of medication were scored as follows: a score of 1 was assigned to colchicine; 2 to anti-TNF; 3 to steroids; and 4 to immunosuppressive drugs. If a patient was taking 2 or more medications, the highest score was assigned to the type of medication.

The diagnosis of AP was confirmed by clinical and radiographic examinations. All types of AP, including asymptomatic AP, symptomatic AP, acute apical abscess, and chronic apical abscess were considered. All radiographic images were obtained using the same imaging system (Planmeca Promax; Planmeca, Helsinki, Finland). If the periapical tissue was normal or had minimal changes in bone structure, the related tooth was classified as healthy (PAI 1 and 2), according to the periapical index (PAI).[19] In the event of a lost periodontal ligament or periodontitis with exacerbating features (PAI 3, 4 and 5), the tooth was classified with a periapical pathology.[20] The PAI score for multi-rooted teeth was determined based on the highest score observed across all roots.[21]

Digital panoramic radiographs were taken and analyzed by an endodontist and an experienced dentist (Fig. 1,2). Both observers were blinded to the study groups. In cases of disagreement, the analysis was repeated. After the radiographic analysis, pulp sensitivity, percussion and

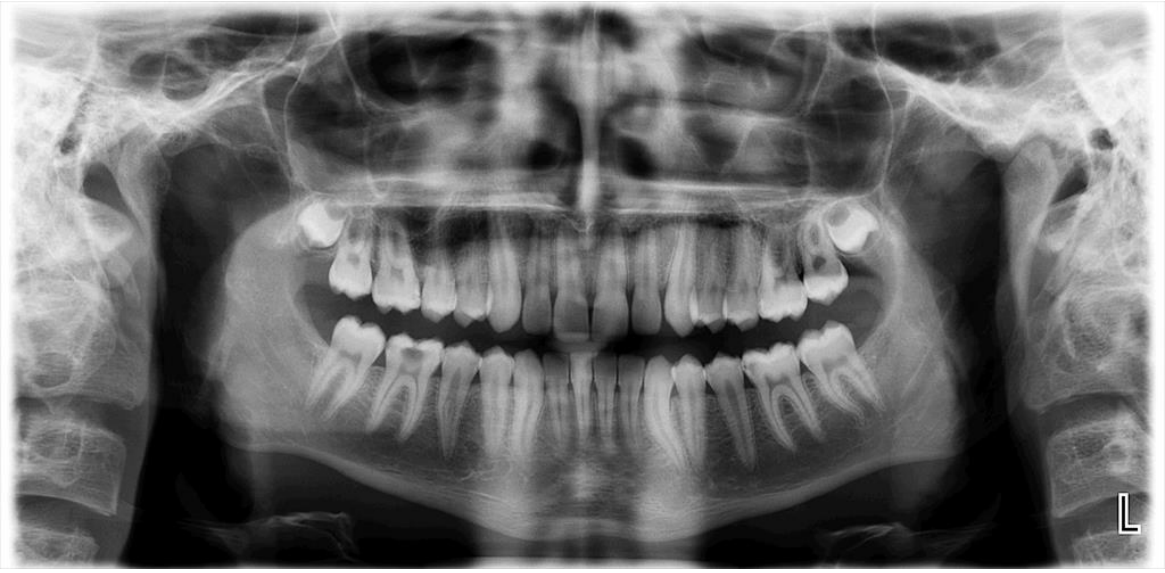

Fig. 1. Panoramic radiograph of a patient included in the control group

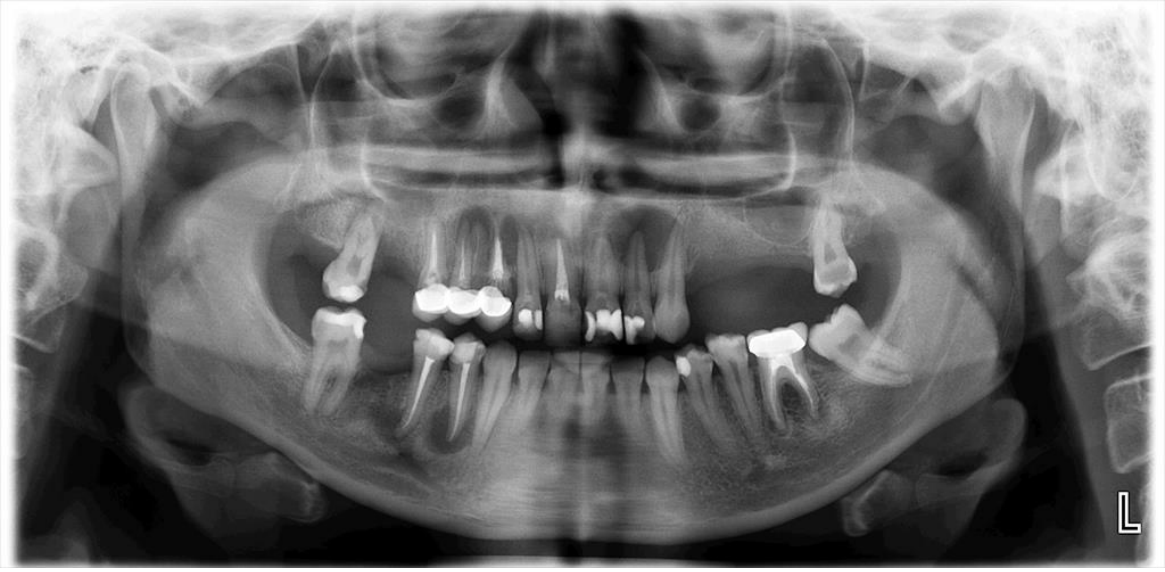

Fig. 2. Panoramic radiograph of a patient included in the experimental group

palpation tests were performed to confirm the clinical diagnosis of AP. Patients with at least 1 tooth exhibiting signs of AP were included in the AP group.

The following data was recorded for all patients: the number of patients with AP; the number of patients with root canal-treated (RCT) teeth; and the number of patients with RCT teeth and AP.

Statistical analysis

The statistical analysis was conducted using the IBM SPSS Statistics for Windows software, v. 20 (IBM Corp., Armonk, USA). The χ^2 test was performed to determine the differences between the BD group and the control group. A univariate analysis of potential predictors of BD and AP, as well as RCT teeth and RCT teeth with AP, was carried out using the Mann–Whitney *U* test. Furthermore, a model was constructed for the logistic regression analysis to better understand the relationship between cases with BD and AP, RCT teeth, and RCT teeth with AP. A p-value <0.05 was considered statistically significant.

Results

A total of 98 individuals with 2,051 teeth (1,026 in the BD group and 1,025 in the control group) were evaluated. While 32 patients were diagnosed with AP in the BD group, only 12 patients were diagnosed with AP in the control group. The prevalence of AP was significantly higher in the BD group (65.3%) compared to the control group (24.4%) (odds ratio = 5.804, $p < 0.001$) (Table 1).

Table 1. Distribution of the analyzed variables in patients with Behçet's disease and the control group

Variable		Experimental group	Control group	*p*-value	Odds ratio
Patients, *n*		49	49	–	–
Mean age [years] *M* ±*SD*		34.46 ±9.2	34.46 ±9.2	1.00	–
Gender, *n*	female	31	31	–	–
	male	18	18	–	–
Patients with AP, *n* (%)	present	32 (65.3)	12 (24.4)	<0.001*	5.804
	absent	17 (34.7)	37 (75.6)		
Patients with RCT teeth, *n* (%)	present	30 (61.2)	28 (57.2)	0.837	1.184
	absent	19 (38.8)	21 (42.8)		
Patients with RCT teeth+AP, *n* (%)	present	9 (18.3)	6 (12.2)	0.575	1.613
	absent	40 (81.7)	43 (87.8)		

M – mean; *SD* – standard deviation; AP – apical periodontitis; RCT – root canal-treated; patients with AP – at least 1 tooth with AP; patients with RCT teeth – at least 1 RCT tooth; patients with RCT teeth+AP – at least 1 RCT tooth and AP; * statistically significant ($p < 0.05$).

At least 1 RCT tooth was found in 30 (61.2%) and 28 (57.2%) patients in the BD and control groups, respectively. The observed difference in the number of RCT teeth between the BD and control groups was not statistically significant ($p > 0.05$). At least 1 RCT tooth with AP was identified in 9 (18.3%) and 6 (12.2%) BD and control patients, respectively. The difference between the groups in terms of the presence of RCT teeth with AP was not statistically significant ($p > 0.05$) (Table 1).

The intragroup analysis revealed a higher prevalence of AP in male BD patients compared to female patients ($p < 0.05$) (Table 2). Additionally, the prevalence of patients with AP was significantly higher in the severe group than in the mild and moderate groups ($p < 0.05$). There was no statistically significant difference between the mild and moderate groups in terms of the AP prevalence ($p > 0.05$). No statistically significant difference was observed in terms of age, the duration of the disease, or the type of medication among patients with BD ($p > 0.05$). To better understand the relative influence of the predictors, a logistic regression analysis was performed (Table 3). The constructed model demonstrated that BD activity was a predictor of AP ($p < 0.05$).

Table 3. Results of the logistic regression analysis in patients with Behçet's disease

Variable		B*	Standard error	Beta	*p*-value
Type of medication	score 1	–	–	–	0.581
	score 2	1.001	1.232	2.721	0.417
	score 3	0.041	1.410	1.042	0.977
	score 4	–0.808	0.813	0.446	0.320
Behçet's disease activity	mild	–	–	–	0.077
	moderate	–0.391	0.727	0.676	0.590
	severe	2.291	1.150	9.886	0.046*
Duration of the disease		0.034	0.051	1.034	0.513
Constant		0.757	0.686	2.132	0.270

* statistically significant ($p < 0.05$, χ^2 test).

Table 2. Distribution of the analyzed variables in patients with Behçet's disease

Variable		AP			RCT teeth			RCT teeth+AP		
		present	absent	*p*-value	present	absent	*p*-value	present	absent	*p*-value
Gender, *n*	female	15	16	0.010*	22	9	0.080	7	24	0.454
	male	15	3		8	10		2	16	
Age [years] *M* ±*SD*		33.8 ±8.5	35.5 ±10.5	0.813	34.7 ±9.9	34.1 ±8.4	0.935	33.4 ±8.1	34.7 ±9.6	0.675
Behçet's disease activity, *n* (%)	mild	11 (52.3)[a]	10 (47.7)[a]	0.040*	13 (61.9)	8 (38.1)	0.200	5 (23.8)	16 (76.2)	0.669
	moderate	8 (50.0)[a]	8 (50.0)[a]		12 (75.0)	4 (25.0)		2 (12.5)	14 (87.5)	
	severe	11 (91.6)[b]	1 (8.4)[a]		5 (41.6)	7 (58.4)		2 (16.6)	10 (83.4)	
Type of medication, *n* (%)	score 1	19 (63.3)	11 (36.7)	0.789	19 (63.3)	11 (36.7)	0.705	7 (23.3)	23 (76.7)	0.088
	score 2	3 (75.0)	1 (25.0)		3 (75.0)	1 (25.0)		2 (50.0)	2 (50.0)	
	score 3	2 (66.7)	1 (33.0)		1 (33.3)	2 (66.7)		–	3 (100.0)	
	score 4	6 (50.0)	6 (50.0)		7 (58.3)	5 (41.7)		–	12 (100.0)	
Duration of the disease [months] *M* ±*SD*		8.2 ±6.3	7.1 ±7.3	0.231	7.8 ±7.2	7.7 ±6.0	0.951	6.6 ±4.4	8.0 ±7.1	0.970

* statistically significant ($p < 0.05$, χ^2 test). Different superscript letters show statistical differences between the groups.

Discussion

Systemic disorders can be regarded as modulating factors affecting the progression of an oral infection, rather than etiologic factors.[22,23] The success of root canal treatment is adversely affected in individuals with diabetes and hypertension.[24] Costa et al. revealed that individuals with cardiovascular diseases are more prone to endodontic pathologies.[25] Moreover, patients who had undergone kidney transplantation exhibited a higher prevalence of endodontic pathology compared to healthy individuals.[26] However, this is the first study investigating the relationship between AP and BD based on a range of variables.

Apical periodontitis is associated with an elevated level of cytokines and inflammatory mediators secreted by T cells, including IL-1, IL-2, IL-6, and TNF-α.[27] In addition, the presence of CD57-positive cells in all cases of chronic AP suggests that activated natural killer (NK) cells contribute to the progression of periapical pathologies.[28] Natural killer cells can release cytokines, including IFN-γ and TNF.[29] The bone destruction process in periradicular pathology is regulated by pro-inflammatory cytokines, such as IL-1β, IL-6, IFN-γ, and TNF-α.[30] Th1 cells are mainly effective in the pathogenesis of BD. It has been shown that Th1 cells and cytokines are significantly elevated in individuals with active BD.[31] Interleukin-1, IL-6 and TNF-α are the main cytokines observed in BD patients.[32] Hasan et al. have confirmed the association between NK cells and BD and identified a reduction in NK cells in the peripheral blood of active BD patients.[33] The depletion of NK cells in the peripheral blood of BD patients may reflect an increased homing of these cytotoxic cells to inflammatory sites.[5] Additionally, increased levels of IFN-γ, which are secreted by NK cells, have been reported in individuals with active BD.[31] Therefore, the association between the 2 diseases can be attributed to increased cytokine similarity and the impact of these cytokines on disease progression in both AP and BD.

The present study indicates that the prevalence of AP was higher in male individuals in the BD group. While BD has an impact on both genders, it is more severe among young males.[34–36] The relevant clinical difference between men and women is unclear, but it may be related to hormonal factors, such as testosterone and prolactin.[37,38]

Despite the lack of consensus on a treatment program, colchicine, corticosteroids, anti-TNF agents, and cyclosporine have been demonstrated to effectively control BD symptoms.[39] Drug therapy provides symptom control, reduces inflammation, supports the immune system, provides remission, and increases the patient's quality of life.[40] However, our study did not identify an association between AP and the type of medication used to treat BD. Since no other study has investigated the possible associations between the type of medication and AP, a direct comparison cannot be made.

The study results indicated that there was no statistically significant difference between the groups with regard to the prevalence of RCT teeth and those with RCT teeth and AP. In other words, BD did not affect the maintenance of endodontic treatment. Bone destruction in AP has been related to the Th1 response, which leads to the activation of osteoclasts.[41] The administration of intracanal medication during endodontic treatment can result in successful outcomes, as evidenced by a previous study which indicated that intracanal medication can reduce the levels of cytokines involved in AP.[42]

A limitation of this study is the absence of data regarding the patient's oral hygiene. Oral hygiene may influence the prevalence of AP. Previous research has shown that the incidence of AP is higher among patients with periodontal disease (PD) compared to those without PD.[43] Another study has reported that dental caries and PD were the most prevalent oral health concerns in individuals with rheumatic diseases (73.1%).[44] These dental problems could be attributed to the side effects of medications.[45] Another limitation of the present study is that it was based on an observational cross-sectional design, which only captured data at a single point in time. However, the results of the present study provide baseline information for prospective studies with larger sample sizes. Finally, further controlled clinical trials are required to elucidate the role of AP in BD.

Conclusions

Behçet's disease was shown to be significantly associated with an increased prevalence of AP. It can be concluded that, among all variables, BD activity was the most effective predictor of AP. Individuals with severe BD may be more prone to developing AP in comparison to those with mild and moderate BD activity. No statistically significant difference was observed regarding the prevalence of RCT teeth with AP between the BD and control groups. Thus, it can be concluded that BD did not affect the success rate of root canal treatment. Further prospective studies are required to confirm the relationship between BD and AP.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Atatürk University (decision No. 71-09/2022).

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Meltem Sümbüllü https://orcid.org/0000-0002-2647-7988
Ayhan Kul https://orcid.org/0000-0003-1313-9469
Ertuğrul Karataş https://orcid.org/0000-0002-8145-8763
Meltem Memiş https://orcid.org/0000-0001-7393-5729

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Original paper

Smokeless tobacco- and quid-associated localized lesions of the oral cavity: A cross-sectional study from a dental institute

Virender Gombra[1,A,D,F], Mandeep Kaur[2,D–F], Shamimul Hasan[2,B,F], Shahnaz Mansoori[2,B,C]

[1] Faculty of Dentistry, Jamia Millia Islamia, New Delhi, India
[2] Department of Oral Medicine and Radiology, Faculty of Dentistry, Jamia Millia Islamia, New Delhi, India

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):687–696

Address for correspondence
Virender Gombra
E-mail: vgombra@jmi.ac.in

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on May 23, 2022
Reviewed on July 21, 2022
Accepted on July 27, 2022

Published online on October 10, 2024

Abstract

Background. There is a well-established link between the use of smokeless tobacco (ST) and the development of oral cancer. This study was conducted to evaluate the impact of tobacco use, quid use, and other adverse habits related to smoking and alcohol consumption on ST-induced localized lesions.

Objectives. The aim of the study was to examine the demographic data, frequency and contact duration of ST on the lesion, as well as to conduct a clinical evaluation of these parameters.

Material and methods. A total of 13,442 patients who had been experiencing oral and dental symptoms for a period of at least 6 months were screened. Of these, 334 patients were diagnosed with ST- or quid-induced localized lesions and had a positive history of ST or quid use. A structured questionnaire was employed to conduct interviews with participants regarding their use of ST and other adverse habits, including smoking and alcohol consumption. Other information related to the use of ST or quid and clinical findings were also recorded, along with the patients' demographic details. A statistical analysis was carried out using the χ^2 test and the regression analysis.

Results. The overall prevalence of ST-induced localized lesions was found to be 2.48%. In the study population, the majority of participants (58.7%) reported a habit of using khaini, while 26.8% reported using gutkha. The study found significant differences in the severity of ST-induced localized lesions and contact duration, frequency of the habit, and the presence of additional habits such as smoking and/or alcohol consumption. Based on this study, we proposed a modified Greer and Poulson's classification of ST-induced lesions, dividing them into 4 clinical types.

Conclusions. Smokeless tobacco-induced localized lesions frequently remain asymptomatic, with patients unaware of their presence. Other adverse habits, including smoking and alcohol consumption, as well as increased ST contact duration were associated with the development of more severe ST-induced localized lesions.

Keywords: mucosal lesions, snuff, smokeless tobacco, chewing tobacco

Cite as
Gombra V, Kaur M, Hasan S, Mansoori S. Smokeless tobacco- and quid-associated localized lesions of the oral cavity: A cross-sectional study from a dental institute. *Dent Med Probl.* 2024;61(5):687–696. doi:10.17219/dmp/152439

DOI
10.17219/dmp/152439

Background

Smokeless tobacco (ST) refers to a range of products, including chewing tobacco, snuff, betel quid with tobacco, and paan.[1,2] The use of ST has been associated with the development of oral potentially malignant disorders (OPMDs) and oral cancer. Oral potentially malignant disorders encompass all lesions and conditions exhibiting morphological changes with an increased potential for malignant transformation.[3]

Tobacco use has been linked to the development of OPMDs, including leukoplakia (white lesions) and erythroplakia (red lesions). Leukoplakia is the initial oral manifestation in over 33% of cases of oral cancer. Thus, white lesions may serve as an early sign for precancerous lesions and should be monitored for an early diagnosis of cancer.[4]

The high incidence of oral cancer in India reflects the pervasive use of tobacco products.[5] In North India, betel quid with tobacco, zarda, gutkha, khaini, and toombak are particularly prevalent and are used by keeping them directly in the buccal and labial vestibules.[6,7] According to Naveen-Kumar et al., 40.64% of patients with oral lesions had a positive history of ST chewing.[8] The most commonly used ST includes chewing or spit tobacco in the form of leaves. Spitless tobacco is finely milled and dissolves orally; snuff tobacco is a fine powder. The dry form is inhaled, while the moist form is placed intraorally.[9] Quid may be used as a mixture of areca nut, catechu, and slaked lime with tobacco (gutkha) or without tobacco (pan masala).[2] Quid is placed intraorally and remains in mucosal contact for a period of time.[1] The slaked lime releases an alkaloid and reactive oxygen species from the areca nut, which produces euphoria. From a clinical perspective, ST-associated lesions are less characteristic and differ from those caused by smoking. Smokeless tobacco contains numerous carcinogens, including polonium-210, tobacco-specific nitrosamines (TSNAs), volatile aldehydes, and polycyclic aromatic hydrocarbons.[5] Nicotine, an alkaloid, is primarily responsible for addiction.[8] The amount of tobacco used in snuff per day is about 10–15 g. It has been demonstrated that orally absorbed nicotine remains in the blood for a longer period of time.[5]

Tobacco pouch keratosis (TPK) is also known as snuff dipper's keratosis or smokeless tobacco keratosis. It is a keratotic mucosal lesion resulting from chronic contact with ST. The mucosal surface affected by ST contact may appear as gray, gray-white, white, translucent, corrugated, or wrinkled. The stretched mucosa may present as a fissured or rippled pouch, which may become leathery or nodular in long-term tobacco users.[2] Very few studies have investigated ST-associated localized lesions. The present study sought to evaluate the impact of ST use on the oral cavity. Such lesions can evolve into OPMDs. Thus, early diagnosis and management of ST-associated lesions is essential.

Objectives

The aim of the study was to evaluate the awareness of the lesion, the deleterious effect of the use of a specific type of ST, and the concomitant effect of smoking and alcohol intake on localized ST-associated lesions. The demographic data, including the frequency of use, contact duration, clinical appearance, and prevalence of ST lesions, was analyzed in patients who presented to the outpatient department (OPD) of the Department of Oral Medicine and Radiology (Jamia Millia Islamia, New Delhi, India). The study provides information about the impact of the usage of different types of ST on the oral mucosa.

Material and methods

Study population

This analytical cross-sectional study was conducted at the Department of Oral Medicine and Radiology (Faculty of Dentistry, Jamia Millia Islamia, India). The study was approved by the Institutional Ethical Committee of the Faculty of Dentistry, Jamia Millia Islamia (approval No. 15/12/50/JMI/IEC/2015), prior to its commencement. The study population consisted of individuals who attended the OPD between February 2016 and July 2016. Both male and female patients aged 15 years and above were considered for inclusion in the study. The sample size was calculated based on the retrospective OPD data from the register. The prevalence of ST-associated localized lesions was found to be 4%, based on the analysis of data from 20 randomly selected days. The sample size was calculated using nMaster software v. 2 (CMC Vellore, Vellore, India). Based on the assumption of a 4% prevalence of TPK in the study population, an absolute precision of 2.5% and a 95% confidence interval (*CI*), a sample size of 236 was found to be sufficient. The study population included individuals aged 15 and above who had the habit of using ST in any form (khaini, gutkha, paan, spitless tobacco, gutkha with lime, supari with tobacco) and who had been clinically diagnosed with localized tobacco-associated lesions. Only those who provided informed consent for participation in this research and who agreed to the use of the acquired data for research writing purposes were enrolled. Patients who were not willing to consent to participation in the study and those who did not present with ST-associated localized lesions were excluded. A total of 13,442 patients with a range of oral and dental symptoms were screened between February 2016 and July 2016. Patients with a positive history of keeping tobacco quid intraorally were clinically evaluated for the presence of ST- or quid-associated localized lesions. Based on this assessment, a clinical diagnosis of habit-associated lesions was given. The lesions were evaluated in accordance with the Greer and Poulson's classification.[10]

A total of 334 patients were diagnosed clinically with ST-associated lesions. Of these, 269 participants satisfied the inclusion and exclusion criteria for the study (Fig. 1).

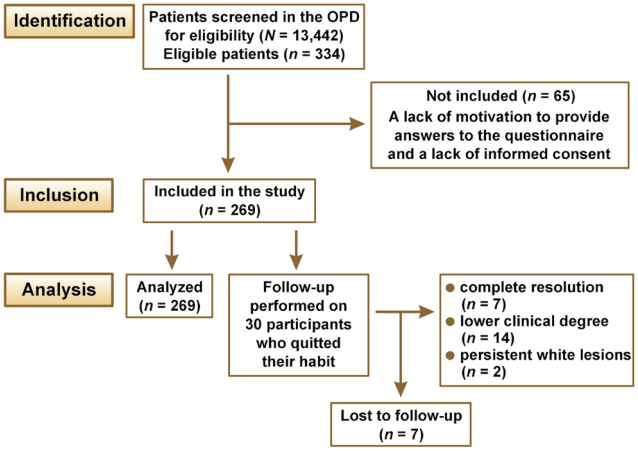

Fig. 1. Study enrolment flowchart
OPD – outpatient department.

Screening program

All individuals who met the eligibility criteria were informed about the nature of the study. A written consent form was provided to each participant after explaining the purpose and process of the research. An intraoral evaluation of the mucosa was conducted using diagnostic instruments and artificial light. The clinical examination of ST-associated localized lesions was performed by trained dental professionals. The screening tool for the diagnosis was based on a clinical evaluation of the lesions in conjunction with a positive history of keeping quid intraorally. The interviewer used a structured questionnaire for the evaluation of ST-related habits and other adverse habits associated with smoking and alcohol consumption. The demographic details, habit-associated history and clinical information were recorded on a proforma. Tobacco pouch keratosis lesions were classified into 3 types according to the Greer and Poulson's classification system (Table 1).[10,11]

Table 1. Greer and Poulson's classification for snuff dipper's lesions[8–10]

Degree of lesion	Description
Degree 1	A superficial lesion with a color similar to that of the surrounding mucosa, with slight wrinkling and no obvious thickening.
Degree 2	A superficial whitish-reddish lesion with moderate wrinkling and no obvious thickening.
Degree 3	A red or white lesion with intervening furrows of normal mucosal color and obvious thickening and wrinkling.

Statistical analysis

The statistical analysis was conducted using the IBM SPSS Statistics for Windows software, v. 16.0 (SPSS Inc., Chicago, USA). The influence of tobacco type and intraoral lesion grading was evaluated using the χ^2 test, with p-values indicating significance at $p < 0.05$. A binary logistic regression analysis was used to analyze the relationship between various covariates and the staging of ST-associated lesions. The dependent variable, namely the stage of TPK, was dichotomized into 2 categories: stage 1; and stage 2/stage 3. All covariates were adjusted for age and sex.

Results

Age and sex profile of the study population

The age and sex distribution of the total study population revealed that 94.8% of patients were male. The patients were classified into 5 age groups (Table 2). The lowest prevalence of lesions was observed in the 15–20 age group. The remaining age groups showed a prevalence of 23–25%.

Table 2. Age and sex distribution of the study population

Variable		Frequency, *n* (%)
Age [years]	15–20	8 (3.0)
	21–30	64 (23.8)
	31–40	66 (24.5)
	41–50	64 (23.8)
	>50	67 (24.9)
Sex	male	255 (94.8)
	female	14 (5.2)

Habit profile of the study population

Most of the ST-associated lesions were asymptomatic, and only 12.6% of participants were aware of lesions at the ST contact site. None of the patients reported any oral lesions associated with tobacco quid use. Sixteen percent of the participants reported a burning sensation at the lesion site. All participants had presented for treatment of an additional orodental complaint, and none had previously consulted with a healthcare practitioner regarding lesions at the quid site. The tobacco types were classified into 6 groups (Table 3). The highest number of grade 3 cases reported a history of using khaini. The χ^2 test demonstrated a statistically significant correlation between the grading of the lesion and the tobacco type habit (Table 4).

Table 3. Habit profile of the study population

Awareness and habit type		Frequency, *n* (%)
Awareness of the presence of lesion	no	235 (87.4)
	yes	34 (12.6)
ST type usage	gutkha	72 (26.8)
	khaini	158 (58.7)
	tobacco	25 (9.3)
	pan masala	1 (0.4)
	pan masala + tobacco	11 (4.1)
	gutkha + lime	2 (0.7)
Other adverse habits	smoking	153 (56.9)
	alcohol consumption	110 (40.9)

ST – smokeless tobacco.

Table 4. Association between different types of ST usage and the grading of ST-associated lesions

ST type	Grading			Total
	grade I	grade II	grade III	
Gutkha	37 (51.4)	29 (40.3)	6 (8.3)	72 (100.0)
Khaini	104 (65.8)	28 (17.7)	26 (16.5)	158 (100.0)
Tobacco	13 (52.0)	11 (44.0)	1 (4.0)	25 (100.0)
Pan masala	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)
Pan masala + tobacco	4 (36.4)	7 (63.6)	0 (0.0)	11 (100.0)
Gutkha + lime	0 (0.0)	0 (0.0)	2 (100.0)	2 (100.0)
Total	159 (59.1)	75 (27.9)	35 (13.0)	269 (100.0)
p-value	<0.001*			

* statistically significant ($p < 0.05$, χ^2 test for different types of ST usage). Data presented as frequency (percentage) (*n* (%)).

A positive history of smoking was reported by 56.9% of the participants, and 40.9% of patients gave a positive history of alcohol consumption. The χ^2 test demonstrated a statistically significant difference in the grading of lesions based on the ST usage, smoking and alcohol consumption. The combination of alcohol consumption, smoking and ST usage was associated with a higher prevalence of grade II and grade III lesions compared to ST-associated lesions (Table 5).

Table 5. Association between the number of habits and the grading of ST-associated lesions

Number of habits	Grading			Total
	grade I	grade II	grade III	
1 habit (only ST)	86 (74.1)	16 (13.8)	14 (12.1)	116 (100.0)
ST & smoking tobacco	25 (58.1)	18 (41.9)	0 (0.0)	43 (100.0)
ST & smoking tobacco & alcohol	48 (43.6)	41 (37.3)	21 (19.1)	110 (100.0)
Total	159 (59.1)	75 (27.9)	35 (13.0)	269 (100.0)
p-value	<0.001*			

* statistically significant ($p < 0.05$, χ^2 test for additional adverse habits). Data presented as *n* (%).

Frequency of ST use and contact duration profile

Most of the participants reported using quid 1–4 times per day, followed by 5–9 times per day (Table 6). The majority of patients kept quid at the contact site for 5–10 min (Table 7). Most of the participants described their habit duration as 0–5 years, followed by 5–10 years (Table 8). The χ^2 test revealed a statistically significant difference between the clinical grading of the lesion and the frequency of ST use, contact time, and the duration of the habit.

Table 6. Association between the frequency of ST use and the grading of ST-associated lesions

Frequency of ST use [times/day]	Grading			Total
	grade I	grade II	grade III	
1–4	93 (69.9)	39 (29.3)	1 (0.8)	133 (100.0)
5–9	41 (40.6)	28 (27.7)	32 (31.7)	101 (100.0)
10–15	23 (71.9)	7 (21.9)	2 (6.3)	32 (100.0)
>15	2 (66.7)	1 (33.3)	0 (0.0)	3 (100.0)
Total	159 (59.1)	75 (27.9)	35 (13.0)	269 (100.0)
p-value	<0.001*			

* statistically significant ($p < 0.05$, χ^2 test for the frequency of ST use). Data presented as *n* (%).

Table 7. Association between the duration of contact with ST and the grading of ST-associated lesions

Contact duration [min]	Grading			Total
	grade I	grade II	grade III	
0–5	47 (92.2)	4 (7.8)	0 (0.0)	51 (100.0)
5–10	57 (46.7)	36 (29.5)	29 (23.8)	122 (100.0)
10–15	33 (71.7)	13 (28.3)	0 (0.0)	46 (100.0)
>15	22 (44.0)	22 (44.0)	6 (12.0)	50 (100.0)
Total	159 (59.1)	75 (27.9)	35 (13.0)	269 (100.0)
p-value	<0.001*			

* statistically significant ($p < 0.05$, χ^2 test for the duration of contact with ST). Data presented as *n* (%).

Table 8. Association between the duration of ST usage and the grading of ST-associated lesions

Habit duration [years]	Grading			Total
	grade I	grade II	grade III	
0–5	70 (70.7)	29 (29.3)	0 (0.0)	99 (100.0)
5–10	48 (67.6)	16 (22.5)	7 (9.9)	71 (100.0)
10–15	18 (51.4)	7 (20.0)	10 (28.6)	35 (100.0)
15–20	7 (18.9)	19 (51.4)	11 (29.7)	37 (100.0)
>20	16 (59.3)	4 (14.8)	7 (25.9)	27 (100.0)
Total	159 (59.1)	75 (27.9)	35 (13.0)	269 (100.0)
p-value	<0.001*			

* statistically significant ($p < 0.05$, χ^2 test for the duration of ST usage). Data presented as *n* (%).

Site of involvement

Smokeless tobacco-associated lesions were observed unilaterally in 69.0% and bilaterally in 21.0% of the participants. Eighty-four percent of cases were seen in the mandibular arch. The highest prevalence of cases was observed on the buccal mucosa, at 88.5%. A single case was reported in the retromolar mucosa, and no cases were identified on the tongue, floor of the mouth, or palatal region. Most of the lesions were present in the left mandibular vestibule (incisor and premolar region). The majority of the lesions were less than 3 cm in size (86.0%). The color of the lesion was white or grayish-white in 84.5% of cases, mixed red and grayish-white in 13.0%, and red in 2.6% of the study sample. The majority of the lesions (88.5%) had ill-defined margins. A total of 36.0% of the grayish-white lesions were scrapable. The lesion could be removed by pulling from the margin of the lesion, leaving an underlying erosive area. A plaque-like appearance was observed in 49.0% of cases, while 11.0% of the lesions showed a corrugated appearance. Tobacco-associated staining was observed adjacent to the dentition in approx. 68.0% of the lesions, and mucosal staining was seen in 34.0% of the lesions. Erythematous changes were present in 15.0% of the cases (Table 9). According to the classification proposed by Greer and Poulson, 59.1% of the participants demonstrated degree 1, 27.9% exhibited degree 2, and 13.0% displayed degree 3 of the lesions (Fig. 2). Additionally, hard tissue changes manifesting as brownish discoloration were observed in the adjacent dentition due to the contact with the ST.

Of the 269 ST-associated lesions, 41 cases exhibited the presence of other tobacco-associated lesions in the following order: 16 were smoker's palate; 9 were leukoplakia; 9 were leukoplakia with smoker's palate; 5 were oral submucous fibrosis with leukoplakia; and 2 were oral submucous fibrosis (Fig. 3).

A binary logistic regression analysis was used to analyze the association between habit and the staging of the lesion. The dependent variable, namely the stage of TPK, was dichotomized into 2 categories: stage 1; and stage 2/stage 3. All covariates were adjusted for age and sex. With the exception of the type of ST, all covariates were found to have a significant association with the staging of the study lesion. The results indicated that the odds of developing stage 2/3 of the lesion were 3.27 times higher (odds ratio (*OR*) = 3.270; 95% *CI*: 1.778–6.014) for a 5–9 times/day frequency of ST usage compared to the lower frequencies. The odds of having stage 2/3 were 7.382 times higher (*OR* = 7.382; 95% *CI*: 2.391–22.790) for those with a duration of habit greater than 15 years compared to other habit durations (Table 10).

A total of 30 participants were randomly selected and followed for 6 weeks to observe the effects of quitting the adverse habit. Seven patients were lost to follow-up.

Table 9. Clinical evaluation of the site of involvement

Variable		Frequency [%]
Unilateral/bilateral	unilateral	69.0
	bilateral	21.0
Arch involvement	mandibular arch	84.0
	maxillary arch	26.0
Maxillary or mandibular vestibule involvement	maxillary anterior (incisor region)	1.4
	mandibular anterior (incisor region)	14.0
	maxillary left and right side	2.9
	left maxillary posterior (premolar, molar region)	0.7
	left mandibular posterior (premolar, molar region)	55.7
	right maxillary posterior (premolar, molar region)	0.7
	right mandibular posterior (premolar, molar region)	7.4
	mandibular left and right side	17.9
Mucosal site involvement	buccal mucosa	88.5
	mucobuccal fold	71.0
	labial mucosa	31.2
	gingiva	30.9
	retromolar region	0.4
	floor of the mouth	0.0
	tongue	0.0
Size	<3 cm	86.0
	>3 cm	14.0
Color	grayish-white/white	84.5
	grayish-white and red	13.0
	red/velvet	2.6
Burning at lesion site	present	16.0
	not present	84.0
Margin	ill-defined	88.5
	defined	11.5
Scrapable	yes	36.0
	no	84.0
Corrugated appearance	present	11.0
	absent	89.0
Plaque-like appearance	present	49.0
	absent	51.0
Erosive lesion	present	15.0
	absent	85.0
Extrinsic staining	dentition	68.0
	mucosal staining	34.0
Gingival recession at the contact site		42.0
Discolored teeth	present	68.0
	absent	32.0

The follow-up of the remaining 23 participants demonstrated that in 14 lesions, the clinical severity decreased following a reduction in the habit. Additionally, 7 cases

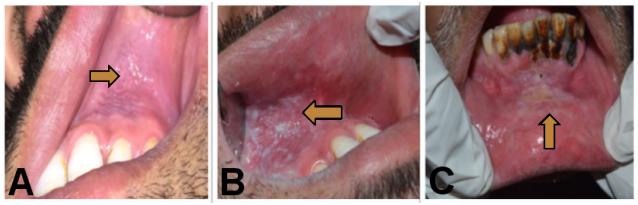

Fig. 2. Degree 1 lesion on the labial mucosa (A), degree 2 lesion on the buccal mucosa (B) and degree 3 lesion involving the gingiva and buccal mucosa (C)

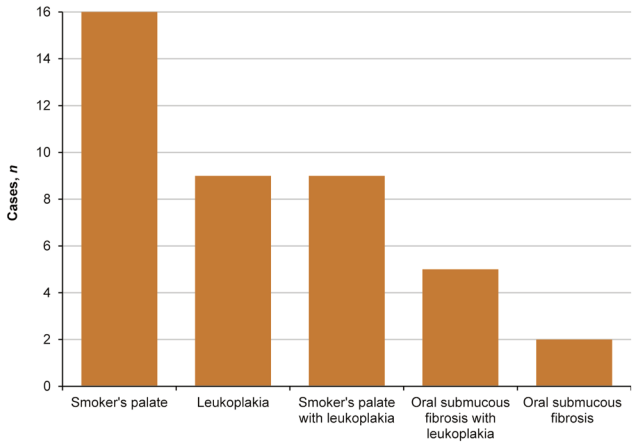

Fig. 3. Other smokeless tobacco (ST)-associated lesions identified in the participants of the study

showed complete resolution of the lesions, while 2 cases demonstrated persistence of white lesions at the lesion site even after quitting the habit for 6 weeks (Fig. 4,5).

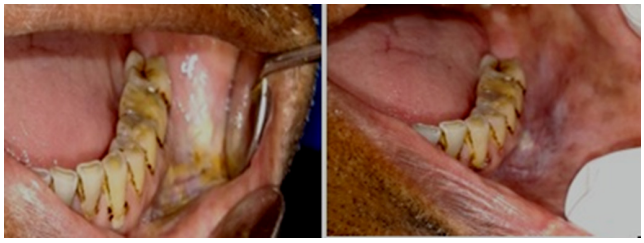

Fig. 4. Khaini-induced lesion (A) and its clinical appearance at the 6-week follow-up after the cessation of the habit (B) – case 1

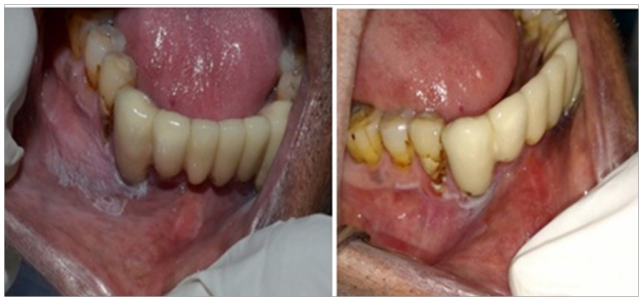

Fig. 5. Khaini-induced lesion (A) and its clinical appearance at the 6-week follow-up after the cessation of the habit (B) – case 2

Table 10. Impact of different predictor variables on the grading of ST-associated localized lesions

Variable		*p*-value	Adjusted *OR*[a]	95% *CI* of *OR*	
				lower limit	upper limit
Tobacco type	gutkha	0.227	1	–	–
	khaini	0.218	0.678	0.365	1.258
	tobacco	0.737	1.189	0.432	3.276
	pan, pan masala + tobacco, gutkha + lime	0.316	1.890	0.545	6.557
Frequency of quid use [times/day]	0–4	<0.001*	1	–	–
	5–9	<0.001*	3.270	1.778	6.014
	10–15	0.043*	0.354	0.130	0.969
	>15	0.955	1.092	0.052	22.820
Contact duration [min]	0–5	<0.001*	1	–	–
	5–10	<0.001*	12.808	4.133	39.687
	10–15	0.115	2.789	0.778	10.003
	>15	<0.001*	12.288	3.614	41.784
Habit duration [years]	0–5	0.003*	1	–	–
	5–10	0.880	1.056	0.523	2.132
	10–15	0.382	1.466	0.622	3.458
	15–20	0.001*	7.382	2.391	22.790
	20	0.561	0.733	0.258	2.087
Smoking	absent	–	1	–	–
	present	<0.001*	2.814	1.592	4.976
Alcohol consumption	absent	–	1	–	–
	present	0.002*	2.309	1.344	3.965
Number of habits	1 habit (only ST)	0.001*	1	–	–
	ST & smoking tobacco	0.039*	2.365	1.046	5.345
	ST & smoking tobacco & alcohol	<0.001*	2.985	1.634	5.454

[a] adjusted for age and sex; * statistically significant ($p < 0.05$, binary logistic regression analysis); *OR* – odds ratio; *CI* – confidence interval.

Discussion

Globally, oral cancer is the 3rd most common cause of mortality in developing countries and the 8th most common cause in developed countries. The use of tobacco is associated with an increased incidence and mortality rate. In 1978, the World Health Organization stated that the appearance of oral cancer is preceded by other lesions, which may show cellular changes that may develop into malignancy.[12] Smokeless tobacco in various forms is used in many countries in Southeast Asia.[13]

Multiple risk factors of leukoplakia have been identified, including genetic factors, local injury, tobacco use, the presence of Candida species, Epstein–Barr virus, sanguinaria, alcohol consumption, and nutritional deficiencies. Recent studies have also demonstrated that the oral cavity is the most common site for drug-induced cancer in the head and neck region. Oral cancer-associated drugs include immunomodulatory/immunosuppressive and chemotherapeutic agents. However, the mechanism by which these drugs induce oral cancer remains unclear. Tobacco use is considered the main etiological factor in the development of oral cancer.[14,15]

Tobacco use has been recognized as a risk factor for the development of OPMDs of the oral mucosa, such as leukoplakia and erythroplakia. The prevalence of these conditions among patients attending a hospital in certain areas of India ranges from 2.5% to 8.4%, respectively. The development of cancer has been demonstrated in up to 17% of cases within a mean period of 7 years after diagnosis.[16] The term "leukoplakia" refers to a clinical diagnosis, and as this lesion is an OPMD, it should be considered a precancerous lesion.[4]

Various management options have been considered for OPMDs, including tobacco cessation, antifungal therapy for Candida-associated leukoplakia, retinoids, topical bleomycin, photodynamic therapy, surgical excision, electrocoagulation, cryosurgery, and laser surgery.[14]

Future treatment options for oral cancer may include the use of different types of extracts from propolis containing ethanol, hexane and their combinations, which showed anticancer activity in tongue cancer cells.[17] Other potential management options for oral cancer include polynucleotides, such as graphene oxide–polyethyleneimine, which has been shown to inhibit tumor growth, peptide-based cancer immunotherapy, and antisense oligonucleotides. They reduce the expression of survivin, the factor responsible for the growth of tumors and their resistance to treatment. Furthermore, antisense oligonucleotides increase the apoptotic rate and make the tumor more sensitive to radiation and chemotherapy.[18]

Smokeless tobacco-associated lesions have been identified as the mucosal lesions most commonly associated with tobacco use. In a study conducted by Joshi and Tailor at Gujarat Hospital in India, 53.1% of cases with an ST habit were identified.[19] In a study by Samatha et al., 38.1% of cases were classified as ST-associated lesions.[12] Previous studies have shown an increased risk of moderate to severe dysplasia with ST compared to the smoked form, as well as a higher incidence of malignant transformation of leukoplakia due to ST in comparison to the smoked form.[13,20]

Tobacco is consumed in a variety of ways, including both smokeless and smoked forms. Previous studies in Scandinavia have demonstrated that moist or loose snuff is the most prevalent form of ST.[21–23] In India, the use of betel quid with tobacco was identified as the most common practice.[13,21] In our study, the most frequently used form of ST was khaini (58.7%), followed by gutkha (26.8%) (Fig. 6). This suggests that the prevalence of different ST habits varies depending on region, potentially due to the availability of the ST products. The severity of the lesion is associated with the extent of ST use.[22]

The occurrence of snuff-induced lesions has been documented in previous studies at a rate of 100% among snuff users.[24] In a hospital-based OPD study, the prevalence of ST-associated lesions was found to be 2.4%.[19] Our study found a prevalence of 2.48% for ST-associated lesions. Joshi and Tailor reported a higher prevalence of ST-associated lesions in males compared to females.[19] In contrast, Samatha et al. found ST-associated lesions to be 24.11 times more prevalent in females compared to males in Nepal.[12] In the present study, the sex distribution of the total study population indicated that 94.8% of cases were observed in males and 5.2% of cases were seen in females. The distribution of cases across age groups was nearly uniform, with only a small number of cases observed in the youngest age group (<20 years).

Most of the participants were unaware of the lesion, and only 16.0% of them reported a burning sensation at the contact site. However, they had never visited a physician in relation to this issue.

In our study, the majority of lesions were observed in the left lower arch, which is in accordance with the findings of Bhandarkar et al.[7] The left lower arch was more commonly involved (55.7%) than the right side, and the lesions were least commonly seen in the maxillary posterior region (0.7%).[12]

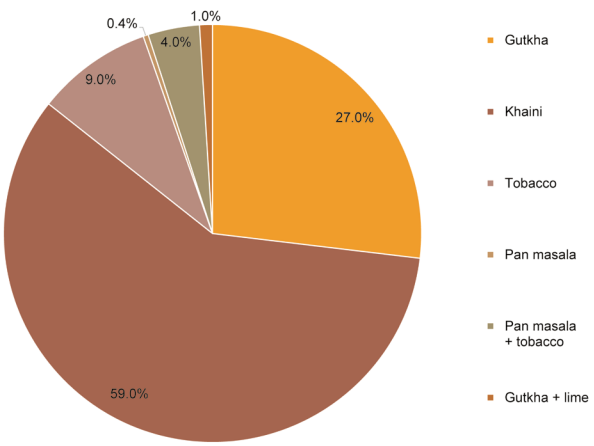

Fig. 6. Smokeless tobacco types used by the participants of the study

The buccal mucosa (88.5%) and the vestibular region (71.0%) were the most frequently affected sites. The lesions were predominantly white or grayish-white in appearance (84.5%), with ill-defined margins. The tongue, palate and floor of the mouth were not affected, suggesting a contact-induced alteration in the mucosa. Since the lower vestibule is the most convenient site for keeping the quid, it is unsurprising that it is the most commonly affected region in our study, as reported by Samatha et al.[12] In a study conducted in Nepal by Shrestha and Rimal, the labial vestibule and the floor of the mouth were identified as the most affected sites due to ST.[23] In our study, ST-associated lesions were observed with high frequency in the mandibular region (84.0%) and unilaterally (69.0%). Gingival recession (GR) localized at the contact site was seen in 42.0% of participants. Bhandarkar et al. revealed that the use of ST was associated with a significantly higher risk of developing GR, with a prevalence of 87.5%.[7] The development of recessions is likely attributable to mechanical and/or chemical trauma to the gingiva.[7] Previous studies have also suggested an association between ST and GR.[21,23] Additionally, several studies have yielded inconclusive evidence regarding the association between ST and GR.[2]

Previously, there has been a discrepancy in the terminology used for the classification of ST-associated lesions, as evidenced in studies by Axéll et al., Greer and Poulson, and Tomar and Winn (Table 11).[10,22,24,25] These classifications did not mention clinically scrapable features or loose tags of white lesions. Johnson et al. classified quid-induced localized lesions, irrespective of the presence or absence of tobacco in the quid.[26] These lesions were considered to be equivalent to snuff-induced lesions at the mucosal contact site.[26] Our clinical findings showed the presence of 36.0% of scrapable lesions in degree 2 and 3 lesions, regardless of whether the patient habitually used quid (Fig. 7). As there is no uniform definition and terminology for lesions associated with tobacco and quid, we propose a uniform terminology and definition for ST-associated lesions, with the aim of facilitating communication among clinicians (Table 12). Our findings also revealed that a few lesions presented as ST-associated white non-scrapable lesions persisted even after habit cessation and could possibly represent the progression of the lesion to leukoplakia. These persistent white lesions could be diagnosed at the initial visit or after observing their persistence

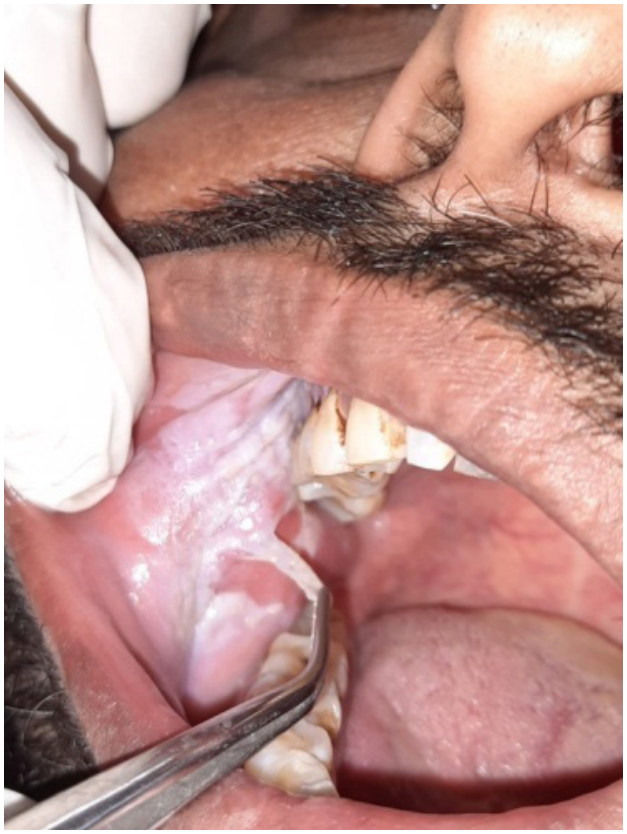

Fig. 7. Khaini-induced lesion (degree 3) with white scrapable features on the buccal mucosa

Table 11. Classifications of mucosal lesions proposed by Axéll, Greer and Poulson, and Tomar and Winn

Study		Description
Axéll 1976[26]	definition	The snuff dipper's lesion is a lesion of the oral mucosa that occurs at the exact site of the regular placing of snuff.
	degree 1	A superficial lesion with a color similar to that of the surrounding mucosa, and with slight wrinkling. There is no obvious mucosal thickening.
	degree 2	A superficial, whitish or yellowish lesion with wrinkling. There is no obvious thickening.
	degree 3	A whitish-yellowish to brown, wrinkled lesion with intervening furrows of normal mucosal color. There is a notable thickening.
	degree 4	A marked, white-yellowish to brown and heavily wrinkled lesion with intervening, deep and reddened furrows and/or a heavy thickening.
Greer and Poulson 1983[10] (modified version of Axéll's classification)	degree 1	A superficial lesion with a color similar to that of the surrounding mucosa, with slight wrinkling and no obvious thickening.
	degree 2	A superficial whitish-reddish lesion with moderate wrinkling and no obvious thickening.
	degree 3	A red or white lesion with intervening furrows of normal mucosal color and obvious thickening and wrinkling.
Tomar and Winn 1999[25]	definition	An ST lesion is a lesion with slight to heavy wrinkling of the mucosa with or without obvious thickening.
	degree 1	Slight, superficial wrinkling of the mucosa. The color of the mucosa may range from its normal hue to pale white or gray. The mucosa does not appear to be thickened.
	degree 2	A distinct whitish, grayish, or occasionally reddish color change. While wrinkling is evident, there is no thickening of the mucosa.
	degree 3	The mucosa is visibly thickened, with distinct whitish or grayish color change. Deep furrows are present within the thickened areas.

Table 12. Modified Greer and Poulson's classification based on our clinical observations

Degree of lesion	Description
Definition	An ST-associated lesion is a localized contact-induced lesion resulting from the use of any form of ST or quid, whether or not accompanied by a habit of smoking and alcohol consumption.
Degree 1	A superficial lesion with a color similar to that of the surrounding mucosa, exhibiting slight wrinkling and no obvious thickening.
Degree 2	A superficial red or white scrapable or non-scrapable lesion with moderate wrinkling and no obvious thickening.
Degree 3	A red or white scrapable or non-scrapable lesion with intervening furrows of normal mucosal color, obvious thickening and wrinkling.
Degree 4	A lesion of any degree with leukoplakia at the contact site.

following the cessation of the habit for 6 weeks. A definitive clinical diagnosis of leukoplakia was considered when a negative result was obtained, even after the elimination of suspected etiologic factors, e.g., mechanical irritation, during a follow-up period of 6 weeks.[27] Leukoplakia is a classic precursor lesion of oral cancer.[28] Such lesions should be managed properly to prevent further severe dysplastic changes.

In our study, 41 participants displayed evidence of additional lesions, attributable to either the smoked or smokeless tobacco form or quid. Many patients reported using other forms of tobacco and quid. It is therefore recommended that a comprehensive history of the patient's habit of smoking, ST use, as well as their alcohol consumption be obtained. This should be followed by a thorough examination to rule out the presence of other potentially malignant disorders and to determine the optimal course of management for the patient.

Gupta et al. observed an increased incidence of oral squamous cell carcinomas in patients who chewed tobacco.[5] Sawyer and Wood also reported an increased risk with an increase in the frequency and duration of the habit.[29] In the present study, the logistic regression analysis identified a significant association between increased duration, frequency of the habit and a higher degree of ST-associated localized lesions. A limitation of this study is that only a clinical diagnosis was considered for the ST-associated localized lesions. Further research should include a histopathological diagnosis of the oral lesions. The present study is limited to examining the prevalence and demographic details of a single dental institute. Thus, it is not possible to generalize the findings, and further research is necessary to document such findings on a larger sample size.

Various research studies have shown the role of different biomarkers in the early diagnosis of oral lesions with a poor prognosis. Studies have demonstrated that the elevated expression of biomarkers such as loss of heterozygosity (LOH) at 3p and/or 9p, Podoplanin, p27 loss, tumor necrosis factor alpha (TNF-α), salivary levels of lactate dehydrogenase (LDH), various interleukins (ILs), osteonectin, and basement membrane protein 40 (BM-40) in oral lesions such as leukoplakia is associated with an increased likelihood of malignant conversion. These markers may be used as an early diagnostic approach for OPMDs.[9,30–32] Further research should be conducted on the molecular analysis of ST-associated localized lesions, which will provide insight into the malignant potential of such lesions at an early stage. The present study also showed a significant association between the severity of the lesion and the presence of an adverse habit of smoking and/or alcohol consumption. The habit of smoking, when present alone or in conjunction with alcohol consumption and ST usage, was associated with an increased risk of clinically severe lesions. The effect of quitting the habit and a concomitant reversal of ST-associated lesions is in accordance with the findings of Donald et al., who used the term "tobacco pouch keratosis" and recommended the complete cessation of the tobacco chewing habit and a follow-up to assess resolution.[9]

Conclusions

Oral potentially malignant disorders resulting from an ST habit were mostly asymptomatic at the initial stage. The majority of patients were unaware of the presence of a lesion and thus did not consult a clinician for the condition. The management is more optimistic when ST-associated localized lesions are diagnosed at an early stage. The establishment of uniform terminology, definitions and classifications is essential for effective communication among clinicians. Based on our clinical findings, we propose a modified Greer and Poulson's definition and classification for ST-associated localized lesions, with the aim of facilitating the understanding of such lesions. The results of our study indicate that an increase in the severity of ST-associated localized lesions is linked to an increase in the frequency and contact duration with the ST or quid, irrespective of the type or form of ST. Additionally, a significant association was observed between the increased clinical stage of the lesion and the additional adverse effects of smoking alone or in conjunction with alcohol. Thus, it is essential that all patients undergo a comprehensive history of adverse habits and regular oral examinations in order to facilitate the early management of ST-associated lesions.

Ethics approval and consent to participate

The study was approved by the Institutional Ethical Committee of the Faculty of Dentistry, Jamia Millia Islamia, New Delhi, India (approval No. 15/12/50/JMI/IEC/2015), prior to its commencement.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Virender Gombra https://orcid.org/0000-0002-4047-6877
Mandeep Kaur https://orcid.org/0000-0002-9290-7341
Shamimul Hasan https://orcid.org/0000-0001-6832-9150
Shahnaz Mansoori https://orcid.org/0000-0002-4087-7734

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Original paper

Changes in self-reported sleep and awake bruxism in relation to the management of temporomandibular disorders ("care as usual") in a specialty clinic population

Thiprawee Chattrattrai[1,2,A–F], Magdalini Thymi[1,A–F], Naichuan Su[3,A,C–F], Frank Lobbezoo[1,A,D–F]

[1] Department of Orofacial Pain and Dysfunction, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, the Netherlands
[2] Department of Masticatory Science, Faculty of Dentistry, Mahidol University, Bangkok, Thailand
[3] Department of Oral Public Health, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, the Netherlands

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):697–704

Address for correspondence
Thiprawee Chattrattrai
E-mail: t.chattrattrai@acta.nl

Funding sources
Thiprawee Chattrattrai was supported with an academic development scholarship by Mahidol University, Bangkok, Thailand.

Conflict of interest
None declared

Acknowledgements
None declared

Received on July 10, 2024
Reviewed on August 27, 2024
Accepted on September 9, 2024

Published online on October 31, 2024

Cite as
Chattrattrai T, Thymi M, Su N, Lobbezoo F. Changes in self-reported sleep and awake bruxism in relation to the management of temporomandibular disorders ("care as usual") in a specialty clinic population. *Dent Med Probl.* 2024;61(5):697–704. doi:10.17219/dmp/193125

DOI
10.17219/dmp/193125

Abstract

Background. The treatment of temporomandibular disorders (TMD) often includes the management of sleep bruxism (SB) and awake bruxism (AB). However, few studies have investigated how SB and AB change after the initiation of the interventions aimed at reducing the activity of masticatory muscles in TMD patients.

Objectives. The aim of the present study was to investigate changes in self-reported SB and/or AB with regard to baseline at 6 weeks after receiving TMD treatment, i.e., counseling alone or counseling combined with any other treatment, and to investigate the association between the type of TMD treatment and changes in self-reported SB and/or AB.

Material and methods. A total of 68 TMD patients were included in this prospective study, and they all received counseling. Thirty-three of the 68 patients received additional treatment, e.g., physical therapy, psychological therapy and/or an oral appliance, beside counseling. The self-reported SB and AB frequency values were obtained from the Oral Behavior Checklist (OBC) questionnaire at baseline (t_0) and at week 6 after receiving treatment (t_1). The frequency of SB and AB was assessed as SB, AB-grinding, AB-clenching, AB-bracing, and AB-combined (i.e., the maximum frequency of all AB types combined). The Wilcoxon signed-rank test was used to compare the SB and AB frequency at t_0 and t_1 in patients who received counseling alone and those who received counseling combined with other treatment. The χ^2 test was used to investigate the association between the type of TMD treatment and changes in SB and/or AB.

Results. The frequency of self-reported SB and all types of AB did not change in patients who received counseling only. In contrast, there was a significant increase in the frequency of AB-bracing and AB-combined between t_0 and t_1 in patients who received counseling combined with other treatment.

Conclusions. No changes in the frequency of self-reported SB and all types of AB were found in patients who received counseling only. However, patients who received counseling combined with other treatment showed a significant increase in the frequency of AB-bracing and AB-combined as compared to baseline.

Keywords: treatment, follow-up, temporomandibular disorders, sleep bruxism, awake bruxism

Introduction

Sleep bruxism (SB) is a masticatory muscle activity during sleep, characterized by rhythmic or non-rhythmic movement, while awake bruxism (AB) is a repetitive masticatory muscle activity during wakefulness, characterized by tooth contact and/or the bracing or thrusting of the lower jaw.[1] Bruxism is not considered a disorder, but rather a behavior.[1] The prevalence of self-reported SB ranges from 8.0% to 31.4%, while the prevalence of self-reported AB ranges from 22.1% to 31.0% in the general adult population.[2] Sleep and awake bruxism have been found to be associated with psychosocial factors, such as stress, depression and anxiety.[3] Moreover, SB and AB are often investigated for their association with temporomandibular disorders (TMD). The term 'temporomandibular disorders' refers to a group of conditions related to the temporomandibular joint (TMJ), masticatory muscles and associated structures.[4] The prevalence of TMD symptoms in the adult population is 10.3–30.7%.[5] Common symptoms of TMD are pain, joint sounds and limited jaw movement.[4] The TMD pain has been found to be associated with possible and definite AB.[6] A study found that a higher frequency of self-reported AB, including tooth grinding and clenching, and the bracing of the jaw, was associated with painful TMD.[7] As for SB, possible SB has been found to be associated with the TMD pain and pain interference with daily life activities,[8] but the association between definite SB and the TMD pain is inconsistent.[6,9] A previous study found that probable sleep and awake bruxism, i.e., SB and AB confirmed via a clinical examination, were associated with pain-related TMD.[10] In addition, another study found that 90% of probable sleep bruxers reported jaw-muscle symptoms, such as pain, tiredness or soreness; however, no association was found between muscle activity measured by electromyography (EMG) and jaw-muscle symptoms.[11]

Temporomandibular disorders constitute a multifactorial condition associated with psychological factors (e.g., stress, depression and anxiety), sleep quality and decreased quality of life (QoL).[12,13] In addition, the pain and fear related to jaw movements have been associated with the decision to seek care for the TMD pain.[14] The management of TMD includes multidisciplinary non-invasive treatment, such as counseling, physical therapy, medications, and oral appliance therapy. Invasive treatment, such as TMJ surgery, are less common, and only performed in selected cases.[4,15] The goals of treatment are pain reduction and the recovery of the jaw function.[4] Given the longstanding notion that SB and AB are viewed as masticatory muscle activities that can overload the masticatory system and contribute to the persistence of the TMD pain, TMD treatment strategies often involve the management of SB and/or AB.[15–17] Counseling, including education and behavioral modification, can be implemented to reduce AB,[18] and has been shown to reduce the TMD pain and improve the jaw function.[15,16,18] In addition, the awareness of having AB might help reduce pain.[16] Sleep bruxism is managed through oral appliances, which aim to reduce the loading of the masticatory system due to the forces exerted while bruxing.[19] Biofeedback treatment has been investigated, as it could reduce a jaw muscle activity during sleep,[20,21] as well as during wakefulness,[16] but has not yet been implemented as part of routine treatment for the TMD pain.[22] Even though SB and AB are common targets in the management of TMD, very few studies have investigated how self-reports of SB and AB change after starting interventions that aim at reducing these masticatory muscle activities in TMD patients.[18,23]

The present study aimed to investigate changes in self-reported SB and/or AB with regard to baseline at 6 weeks after receiving TMD treatment, i.e., counseling alone or counseling combined with any other treatment, and to investigate the association between the type of TMD treatment and changes in self-reported SB and/or AB. We hypothesized that changes in self-reported SB and/or AB are associated with the type of TMD treatment. More specifically, we hypothesized that counseling combined with any other treatment may alleviate self-reported SB and AB to a greater extent than counseling alone.

Methods

Study sample

A prospective cohort study was performed in the specialty Clinic for Orofacial Pain and Dysfunction of Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, the Netherlands, from July 2021 until April 2023.

Patients who were referred to the Clinic for Orofacial Pain and Dysfunction of ACTA were eligible to be enrolled in the study if they met the following inclusion criteria:

– at least 18 years old;
– a diagnosis of the TMD pain and/or dysfunction based on the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD),[24] for which treatment would be initiated; and
– signed informed consent.

There was no exclusion for medical or dental reasons. Patients who did not complete the online questionnaire at week 6 after starting treatment (t_1) and those who did not receive counseling treatment were excluded.

The study was approved by the ACTA Ethics Committee (ref. No. 2021-64846), and followed the principles of the Declaration of Helsinki.

Study procedures

The study comprised 3 phases: baseline (t_0); treatment; and follow-up (t_1). First, the TMD patients completed a set of questionnaires before their first visit to the clinic. Second, following a clinical examination during the initial visit, the clinicians prescribed treatment based on the DC/TMD diagnosis, relevant comorbidities, patient

preferences, and professional judgment. Last, the patients completed an online questionnaire 6 weeks after the start of treatment. Further details regarding the applied materials and methods are provided below.

Baseline (t_0)

As part of usual care, all patients completed a set of diagnostic questionnaires before their first visit to the clinic. These questionnaires referred to demographic variables, i.e., age and sex, as well as the average facial pain intensity (the Graded Chronic Pain Scale (GCPS) questionnaire),[25] depression (the Patient Health Questionnaire-9 (PHQ-9)),[26] somatization (the Patient Health Questionnaire-15 (PHQ-15)),[27] and anxiety (the Generalized Anxiety Disorder-7 (GAD-7)).[28] These questionnaires are part of DC/TMD.[24] During the patients' first visit to the clinic, intra- and extraoral inspection, as well as clinical examinations according to DC/TMD were performed. The DC/TMD diagnoses were collected and categorized into 3 categories: pain; dysfunction; and combined pain and dysfunction. The pain category included the DC/TMD diagnoses of local myalgia, myofascial pain, myofascial pain with referral, arthralgia, and headache attributed to TMD. The dysfunction category included the DC/TMD diagnoses of anterior disk displacement with reduction, TMJ subluxation and degenerative joint disease.

Treatment

Each patient received counseling at baseline. The patients received information about their diagnosis and the etiology of their complaints, as well as treatment advice. In addition, the patients could receive one or more other kinds of treatment: physical therapy (including myofeedback, stretching exercises, relaxation, and the self-massage of masticatory muscles); psychological therapy (pain education and a workshop on stress coping); and/or an occlusal splint (a hard occlusal stabilization splint) if the patients reported SB.[29,30] For the purpose of analysis in this study, the type of treatment was categorized into 2 groups: counseling; and counseling with any other treatment.

Follow-up at 6 weeks after starting treatment (t_1)

Changes in SB and AB after the start of treatment were assessed during the follow-up period by means of a questionnaire containing 11 questions that evaluated 3 domains, namely pain and dysfunction,[31] patient complaints through the patient-specific approach (PSA),[32] together with a complaint improvement question, and the frequency of possible SB and AB.[33] The patients received the questionnaire through e-mail 6 weeks after their initial visit to the clinic.

The frequency of self-reported SB and AB was assessed with the Oral Behavior Checklist (OBC) questions 1, 3, 4, and 6.[33] Self-reported SB was assessed with the OBC question 1, i.e., 'clench or grind teeth when asleep based on any information you may have'. The 5 answer options were: never; <1 night/month; 1–3 nights/month; 1–3 nights/week; and 4–7 nights/week. Self-reported AB was assessed with the OBC items 3, 4 and 6, i.e., 'grind teeth together during waking hours' for the AB-grinding type, 'clench teeth together during waking hours' for the AB-clenching type, and 'hold, tighten or tense muscles without clenching or bringing teeth together' for the AB-bracing type. The answer options ranged from 0 (never) to 4 (always). The highest frequency among these 3 questions was used as the maximum frequency of all self-reported AB types combined, i.e., AB-combined. In this study, changes in self-reported SB and AB between t_0 and t_1 were scored as: 1) not improved, if the self-reported SB or AB frequency at t_1 was higher than or equal to the frequency at t_0; or 2) improved, if the self-reported SB or AB frequency at t_1 was lower than the frequency at t_0.

Sample size calculation

The G*Power 3.1.9.7 software (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower)[34] was used to calculate the sample size based on the Wilcoxon signed-rank test. The power of the study was 80%, and the significance level alpha was 0.05. The effect size was set as 0.5, as we assumed a medium size of difference between the 2 groups. A sample size of 35 patients was required.

Statistical analysis

Age, the average facial pain intensity score, and the depression, somatization and anxiety scores were checked for data distribution using the Shapiro–Wilk test. Baseline characteristics, i.e., age, sex, the TMD diagnosis, the average facial pain intensity, and the depression, somatization and anxiety scores, were compared between the 2 treatment groups using the χ^2 test, the Mann–Whitney U test or Fisher's exact test. Differences in the average facial pain intensity and the frequency of self-reported SB and AB, based on the total number of patients, between t_0 and t_1 were compared using the Wilcoxon signed-rank test.

To investigate changes in the frequency of self-reported SB and/or AB between t_0 and t_1 for each type of treatment, the Wilcoxon signed-rank test was used for the patients with counseling alone and separately for those who received counseling combined with other treatment.

To investigate the association between changes in self-reported SB and AB, i.e., improved vs. not improved, on one hand and the type of TMD treatment on the other hand, we used the χ^2 test.

The Castor electronic data capture (EDC) program (Ciwit B.V., Amsterdam, the Netherlands) was used for the collection of study data, and data analysis was

performed with IBM SPSS Statistics for Windows, v. 27.0 (IBM Corp., Armonk, USA). This study complies with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.

Results

There were 172 patients who met the inclusion criteria at baseline (t_0). Of these, 103 patients who did not complete the online questionnaire at week 6 (t_1) and 1 patient who did not receive counseling treatment were excluded. In total, 68 patients were included in this study. There was a significant difference in age between the included and excluded patients ($p = 0.003$). However, there were no significant differences between the included and excluded patients in other baseline characteristics at t_0: sex ($p = 0.539$); the DC/TMD diagnosis ($p = 0.441$); the average facial pain intensity ($p = 0.406$); depression ($p = 0.979$); somatization ($p = 0.616$); and anxiety ($p = 0.702$). Among the 68 patients, there were no significant differences in the average facial pain intensity and the frequency of self-reported SB and AB between t_0 and t_1 (the average facial pain intensity: $p = 0.076$; SB: $p = 0.781$; AB-combined: $p = 0.180$; AB-grinding: $p = 0.853$; AB-clenching: $p = 0.739$; and AB-bracing: $p = 0.110$). The TMD diagnoses in the TMD-pain group included local myalgia ($n = 5$), myofascial pain ($n = 6$), myofascial pain with referral ($n = 6$), arthralgia ($n = 7$), and headache attributed to TMD ($n = 8$). In the TMD-dysfunction group, the diagnoses included anterior disk displacement with reduction ($n = 6$) and TMJ subluxation ($n = 2$). In the combined group, the diagnoses included local myalgia ($n = 16$), myofascial pain ($n = 9$), myofascial pain with referral ($n = 16$), arthralgia ($n = 26$), headache attributed to TMD ($n = 19$), anterior disk displacement with reduction ($n = 23$), TMJ subluxation ($n = 2$), and degenerative joint disease ($n = 5$). Thirty-five of the 68 patients received counseling only, and 33 patients received counseling and other treatment. The types of TMD treatment are shown in Table 1. There were no differences in baseline characteristics between the patients provided with counseling alone and those who received counseling combined with other treatment (Table 2).

Table 1. Distribution of patients according to the provided type of treatment of temporomandibular disorders (TMD)

Type of TMD treatment	*N* = 68
Counseling only	35 (51.5%)
Counseling combined with other treatment:	33 (48.5%)
– physical therapy	13
– psychological therapy	2
– occlusal splint	8
– splint and physical therapy	3
– splint and psychological therapy	1
– splint and physical therapy and psychological therapy	2
– physical therapy and psychological therapy	1
– physical therapy and GrindCare®[a]	1
– physical therapy and BruxApp[b]	1
– medication	1

[a] GrindCare® – biofeedback device (Medotech, Herlev, Denmark); [b] BruxApp – smartphone application form of ecological momentary assessment (EMA) (WMA Italy, Florence, Italy).

Table 2. Comparison of baseline demographic data between the patients provided with counseling alone ($n = 35$) and those who received counseling combined with other treatment ($n = 33$)

Demographic data		Counseling alone ($n = 35$)	Counseling combined with other treatment ($n = 33$)	Total ($N = 68$)	*p*-value
Age (18–86 years) *M* ±*SD*		48.91 ±15.10	46.73 ±45.92	47.9 ±15.4	0.400[b]
Sex *n* (%)	M	7 (20.0)	4 (12.1)	11 (16.2)	0.378[a]
	F	28 (80.0)	29 (87.9)	57 (83.8)	
DC/TMD diagnosis *n* (%)	pain	9 (25.7)	8 (24.2)	17 (25.0)	0.876[c]
	dysfunction	5 (14.3)	3 (9.1)	8 (11.8)	
	combined pain and dysfunction	21 (60.0)	22 (66.7)	43 (63.2)	
Average facial pain intensity score at baseline (t_0) *Me* (*IQR*)		5 (2–7)	6 (4–7)	5 (3–7)	0.129[b]
Average facial pain intensity score at week 6 (t_1) *Me* (*IQR*)		6 (4–7)	6 (3.5–7)	6 (4–7)	0.700[b]
Depression score *Me* (*IQR*)		5 (3–11)	5 (2–8.5)	5 (2.25–10.75)	0.379[b]
Somatization score *Me* (*IQR*)		9 (6–15)	9 (4–12)	9 (5–14)	0.188[b]
Anxiety score *Me* (*IQR*)		5 (2–8)	3 (1–8)	4 (1–8)	0.206[b]

M – mean; *SD* – standard deviation; *Me* – median; *IQR* – interquartile range; M – male; F – female; DC/TMD – Diagnostic Criteria for Temporomandibular Disorders[24]; [a] χ^2 test; [b] Mann–Whitney *U* test; [c] Fisher's exact test.

Among the patients with counseling alone, the frequency of self-reported SB and all types of self-reported AB did not differ between t_0 and t_1 (Table 3). On the other hand, the frequency of AB-bracing and AB-combined at t_1 was significantly increased among the patients with counseling and other treatment as compared to t_0 (Table 4).

Table 5 shows a significant association between improvement with regard to AB-combined and the type of TMD treatment (p = 0.023). Specifically, 78.6% of patients who reported the alleviation of AB-combined and 73.3% of patients who reported the alleviation of AB-bracing were the patients who received counseling alone. In other words, the patients who received counseling alone were significantly more likely to show improvement than those with the combined treatment.

Table 3. Comparisons between the frequency of sleep bruxism (SB) and awake bruxism (AB) at baseline (t_0) and at 6 weeks after receiving treatment (t_1) among the patients with counseling alone (n = 35) (Wilcoxon signed-rank test)

Type of bruxism	Frequency at t_0	Frequency at t_1	p-value
SB	4 (0–4)	3 (1–4)	0.188
AB-grinding	0 (0–2)	0 (0–2)	0.748
AB-clenching	3 (1–3)	2 (1–3)	0.527
AB-bracing	2 (1–3)	2 (1–3)	0.472
AB-combined	3 (2–4)	3 (2–3)	0.059

Data presented as median (interquartile range) (*Me* (*IQR*)).

Table 4. Comparisons between the frequency of sleep bruxism (SB) and awake bruxism (AB) at baseline (t_0) and at 6 weeks after receiving treatment (t_1) among the patients with counseling and other treatment (n = 33) (Wilcoxon signed-rank test)

Type of bruxism	Frequency at t_0	Frequency at t_1	p-value
SB	4 (1.5–4)	3 (1–4)	0.405
AB-grinding	0 (0–2)	0 (0–1)	0.485
AB-clenching	2 (0.5–3)	2 (1–3)	0.255
AB-bracing	2 (0–3)	3 (2–3)	0.008*
AB-combined	2 (1–3)	3 (2–3)	<0.004*

Data presented as *Me* (*IQR*).

Discussion

The present study aimed to investigate changes in self-reported SB and/or AB with regard to baseline at 6 weeks after receiving TMD treatment, i.e., counseling alone or counseling combined with any other treatment. The results showed that the frequency of self-reported SB and all types of AB did not change in patients who received counseling only. In contrast, in patients who received counseling combined with other treatment, there was a significant increase in the frequency of AB-bracing and AB-combined between baseline and week 6 after receiving treatment. This may imply that patients who received counseling with other kind of treatment became more aware of the presence of AB-combined and AB-bracing after receiving treatment as compared to baseline.

A previous study found that patients who believed that jaw-overuse behaviors like AB might cause jaw pain tended to report a higher frequency of such behaviors as compared to those who believed that there were other reasons for jaw pain.[35] In the present study, 63.6% of patients from the counseling and other treatment group received physical therapy, which could indicate that multiple treatment might increase the awareness of having AB in the patients who received such combined treatment. When patients receive multiple treatment, they may recall and recognize more AB events than they did before treatment. Initially, patients may not be aware of their AB until they receive information about it during counseling. Furthermore, repeated exposure to this information through physical or psychological therapy sessions, for example, may increase patients' awareness of their AB behaviors more than in the case of patients who receive such information only once.

Table 5. Association between changes in sleep bruxism (SB) and awake bruxism (AB) in terms of improvement and the type of treatment of temporomandibular disorders (TMD) (χ^2 test)

Type of bruxism	Improvement	Counseling alone (n = 35)	Counseling combined with other treatment (n = 33)	Total (N = 68)	p-value
SB	not improved	29 (82.9)	24 (72.7)	53 (77.9)	0.314
	improved	6 (17.1)	9 (27.3)	15 (22.1)	
AB-grinding	not improved	27 (77.1)	26 (78.8)	53 (77.9)	0.870
	improved	8 (22.9)	7 (21.2)	15 (22.1)	
AB-clenching	not improved	22 (62.9)	25 (75.8)	47 (69.1)	0.250
	improved	13 (37.1)	8 (24.2)	21 (30.9)	
AB-bracing	not improved	24 (68.6)	29 (87.9)	53 (77.9)	0.055
	improved	11 (31.4)	4 (12.1)	15 (22.1)	
AB-combined	not improved	24 (68.6)	30 (90.9)	54 (79.4)	0.023*
	improved	11 (31.4)	3 (9.1)	14 (20.6)	

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

Thus, increasing patients' awareness would be beneficial for bruxism management, especially for AB.[36] This is in contrast with a previous study finding that counseling and self-management strategies, like self-relaxation, self-massage, stretching exercises, and warm/cold compresses, reduced masticatory muscle pain and AB activity, as measured by surface EMG in female TMD patients after 8 weeks of treatment.[37] Meanwhile, usual-care TMD management did not bring improvement with regard to self-reported SB in a brief (6-week) period as compared to self-reported AB. This is in accordance with a previous study showing that sleep hygiene instruction and relaxation techniques did not reduce SB activity, as measured by polysomnography (PSG), when compared between baseline and 4 weeks after the implementation of these techniques.[38] It might be difficult for patients to recognize SB events without a report from their sleep partner. However, the present study shows that usual-care TMD treatment can affect self-reported AB in a brief period.

The present study found that there were differences in the frequency of AB-combined and AB-bracing between baseline and week 6 after receiving treatment in patients who received counseling and other treatment. In addition, it was found that after 6 weeks of receiving treatment, 78.6% of patients who reported the alleviation of AB-combined and 73.3% of patients who reported the alleviation of AB-bracing were the patients who received counseling alone. Notwithstanding, there was no significant association between the improvement of AB-bracing and the type of treatment, but, based on the borderline *p*-value, it might have some clinical significance. The percentage of the improvement of AB-combined and AB-bracing in the patients with counseling alone was much higher than in the patients with counseling and other treatment: 31.4% vs. 9.1% for AB-combined; and 31.4% vs. 12.1% for AB-bracing. These different percentages may represent some clinical significance, namely that different types of treatment may be associated with the awareness of having AB and the AB-bracing subtype more than SB and other AB subtypes. In the sample size calculation, we focused on the comparison of patients with counseling alone and patients who received counseling with any other treatment between 2 time points. Thus, we required at least 35 patients in each group. However, we had 33 patients in the counseling and other treatment group, which indicates that the sample size might not be sufficient. This small sample size (i.e., insufficient power) may be one of the reasons why the type of treatment was not statistically significant with regard to the improvement of AB-bracing. On the other hand, the frequency of self-reported SB, AB-grinding and AB-clenching was comparable between the patients who received different types of treatment, and between those who improved or did not improve the abovementioned behaviors. Since patients provided with counseling and any other treatment may increase their awareness of AB, they may improve their AB behaviors when we continue monitoring over a longer period than 6 weeks. Future research is needed to investigate this matter.

In this study, there was no significant difference in the average facial pain intensity score between baseline and 6 weeks after receiving treatment. In contrast, a study by Donnarumma et al. showed that counseling and self-management strategies could reduce the TMD pain after 8 weeks of receiving treatment, even though the TMD pain was not significantly different between baseline and at 4 weeks of receiving treatment.[37] Similarly, 8 weeks of exercise treatment brought the alleviation of the TMD pain.[39] Thus, it is suggested that a longer period than 6 weeks is required to observe a reduction in the TMD pain.

Even though the sample size was small, we noticed some changes between the time the patients received their treatment and before the end of treatment. Despite some unexpected trends observed in this study with regard to changes in AB, it is recommended to apply a questionnaire to monitor changes in TMD complaints and oral behaviors in regular care. It is beneficial to have a standardized protocol to monitor SB, AB, TMD, and psychosocial factors along the treatment process, as we are doing in usual care.

The strength of this study is that, first, we assessed self-reported SB and AB at baseline and at 6 weeks after starting treatment. To the best of our knowledge, no study has observed the effect of TMD treatment over a brief period on changes in self-reported SB and AB. A practice-based research network study found that 96% and 46% of dental practitioners considered an occlusal appliance and occlusal adjustment, respectively, as appropriate bruxism management.[40] The present study may encourage clinicians to incorporate other kinds of treatment, like counseling and physical therapy, for patients. Clinicians should inform patients that they may become more aware of their AB activity after receiving physical therapy, and patients should subsequently alleviate their AB activity. Moreover, we used part of the OBC questionnaire to assess self-reported AB, and not only the maximum frequency of AB, but also different aspects of AB activity, i.e., grinding, clenching and jaw bracing.

Limitations

There are some limitations to this study. First, the frequency of SB and AB was obtained from self-report, whereas the gold standard of SB and AB assessment is PSG for SB and EMG combined with ecological momentary assessment (EMA) for AB.[1] Sleep bruxism is reported more frequently when assessed through self-report than with PSG.[41] Therefore, using EMG or PSG to assess SB and AB is recommended. In the meantime, the Standardized Tool for the Assessment of Bruxism (STAB) has been developed to assess SB and AB. Using STAB is recommended for future research that would focus on

evaluating the bruxism status, the etiology of bruxism and comorbid conditions.[42] Second, some patients did not fill out all follow-up questionnaires, which were distributed every 6 weeks after the treatment started. Consequently, we had to include only the 1st follow-up questionnaire. Even though a previous study found that biofeedback could reduce SB and AB events, as measured by EMG, in 3 weeks,[43] confirming the cause-and-effect relationship between TMD treatment and SB and/or AB self-reported changes may require a longer period of time. Although 6 weeks is a short period for a longitudinal study, it has clinical relevance as a usual duration for follow-up. Third, we did not measure psychosocial factors after receiving treatment, so we could not monitor changes in the psychosocial status, especially in the patients who received psychological treatment, i.e. in 6 out of the 33 participants who were provided with multiple treatment. Last, due to a small sample size, we did not perform a regression analysis to assess the association between the type of TMD treatment and each type of SB and AB. Future research may require a larger sample size, and should include baseline characteristics for adjustment when assessing these associations.

Conclusions

No changes in the frequency of self-reported SB and all types of AB were found in patients who received counseling only. However, patients who received counseling combined with other treatment showed a significant increase in the frequency of AB-bracing and AB-combined as compared to baseline.

Ethics approval and consent to participate

The study was approved by the Ethics Committee at the Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, the Netherlands (ref. No. 2021-64846). All participants provided signed informed consent.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Thiprawee Chattrattrai https://orcid.org/0000-0002-3715-1211
Magdalini Thymi https://orcid.org/0000-0002-8544-6495
Naichuan Su https://orcid.org/0000-0001-8034-9410
Frank Lobbezoo https://orcid.org/0000-0001-9877-7640

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Original paper

Polish language adaptation and validation of the Fonseca Anamnestic Index for individuals with temporomandibular disorders

Małgorzata Gałczyńska-Rusin[A–D], Małgorzata Pobudek-Radzikowska[B,D], Agata Czajka-Jakubowska[D–F]

Department of Orthodontics and Temporomandibular Disorders, Poznan University of Medical Sciences, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):705–711

Address for correspondence
Małgorzata Gałczyńska-Rusin
E-mail: m.galczynskarusin@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on October 29, 2023
Reviewed on December 4, 2023
Accepted on December 19, 2023

Published online on October 11, 2024

Abstract

Background. Given the notable prevalence of temporomandibular disorders (TMD) in the Polish population, there is a clear need for the use of simple, reliable questionnaires as screening tools to facilitate the referral of patients to TMD specialists.

Objectives. The aim of the study was to translate and adapt the Fonseca Anamnestic Index (FAI) into Polish and assess its reliability and validity in identifying TMD symptoms.

Material and methods. The Polish adaptation of the FAI (FAI-PL) was developed in accordance with the international guidelines, including the translation and evaluation of the psychometric properties of the questionnaire. Every patient received a standardized assessment, which involved history taking and clinical examination, including the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) and the FAI questionnaire. The psychometric analyses included an evaluation of the questionnaire's reliability and validity, as well as an exploratory factor analysis (EFA).

Results. Of the 122 individuals enrolled in the study, 63.9% were female. The mean age of the participants was 28.1 years (standard deviation (*SD*): 6.3). According to the RDC/TMD standards, 40.9% of patients had no TMD, while the FAI assessment indicated that 27% of patients had no TMD. The Cronbach's alpha coefficient for the FAI-PL was 0.75. The exploratory factor analysis revealed 3 factors, accounting for 55.2% of the total variation. The diagnostic sensitivity of the FAI-PL was 98.6%, while the diagnostic specificity reached a level of 65.3%.

Conclusions. The Polish version of the FAI is a reliable and valid tool for the screening of TMD symptoms in the Polish-speaking population.

Keywords: reliability, validity, translation, temporomandibular disorders, screening

Cite as
Gałczyńska-Rusin M, Pobudek-Radzikowska M, Czajka-Jakubowska A. Polish language adaptation and validation of the Fonseca Anamnestic Index for individuals with temporomandibular disorders. *Dent Med Probl.* 2024;61(5):705–711. doi:10.17219/dmp/177287

DOI
10.17219/dmp/177287

Introduction

Temporomandibular disorders (TMD) are a group of clinical conditions involving pain and dysfunction of the temporomandibular joints (TMJs), masticatory muscles and adjacent tissues.[1] The data on the Polish young adult population based on the Polish version of the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD-PL) questionnaire indicates a wide range of TMD prevalence, with figures varying from 26.5% to 54.0%.[2,3] Given the elevated occurrence of TMD among young adults, there is a clear need for screening instruments to aid Polish general dentists in referring patients appropriately.

Over the years, numerous questionnaires have been developed to assess the multifaceted aspects of TMD, including the severity and frequency of symptoms, functional limitations, psychosocial impact, and treatment outcomes. These questionnaires are designed to provide standardized, validated and reliable measures to capture the subjective experiences of individuals with TMD.[4] One of the primary advantages of using questionnaires in a TMD diagnosis is their ability to gather information directly from the patient. The symptoms of TMD can vary significantly among individuals, and patients' self-reporting plays a crucial role in understanding the severity, frequency and impact of the symptoms.

The RDC/TMD and the updated version, called the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD), are current references for standardizing the diagnosis of functional disorders of the masticatory system for research purposes.[1,5,6] The application of standardized diagnostic criteria facilitates the comparison of test results between different countries.[7] Nevertheless, the use of the RDC/TMD or DC/TMD for clinical selection and population screening is impractical due to the long history taking and extensive testing procedure. Screening questionnaires for TMD must be inexpensive, short, simple, accurate, and preferably completed by patients.[1] It has been documented that TMD have a negative impact on the quality of life, and that initiating treatment leads to an improvement in this area.[8,9]

The Fonseca Anamnestic Index (FAI), introduced by Da Fonseca et al. in 1994, is one of the most extensively utilized TMD screening questionnaires.[10] It presents a straightforward, cost-effective and efficient patient-reported assessment, indicating the presence and intensity of TMD symptoms.[11] Due to its simplicity, quick administration and affordability, the FAI is highly recommended for screening individuals with TMD symptoms.[12]

To enable effective cross-study comparisons, there is a need for a concise and user-friendly patient-reported instrument that is both reliable and valid for investigating the epidemiology of TMD.

A comprehensive investigation has yet to be conducted to evaluate the psychometric properties of the Polish version of the FAI (FAI-PL). Given this lack of research, the present study had a dual purpose. Firstly, it aimed to translate the FAI into Polish in accordance with the established guidelines proposed by Beaton et al.[13] Secondly, the study sought to assess the reliability and evaluate the structural, convergent, content, and face validity of the FAI-PL through a comprehensive statistical analysis.

Material and methods

The minimum sample size was set at 100 participants, based on the recommended ratio of 10 subjects per item in the measurement.[14] Participants were recruited from individuals presenting for medical examination and treatment at the Department of Orthodontics and Temporomandibular Disorders at Poznan University of Medical Sciences in Poland. The group consisted of individuals who were seeking either orthodontic assessments or consultations regarding symptoms associated with the masticatory muscles or the TMJ. Individuals aged 18 years and above were eligible to participate in the study. Patients with rheumatoid diseases, individuals who had recently experienced facial trauma, those currently using muscle relaxants and non-steroidal anti-inflammatory drugs (NSAIDs), as well as patients with muscular and neurological disorders were excluded from the study.

All participants underwent a standardized history taking and clinical examination. The patients were examined in accordance with the RDC/TMD-PL Axis I and completed the FAI-PL questionnaire.

The study was approved by the Research Ethics Committee at Poznan University of Medical Sciences, Poland (protocol No. 522/21). Prior to their participation in the study, all participants were duly informed and provided written consent.

Translation

The translation process of the FAI involved 5 stages and adhered to the widely accepted guidelines presented by Beaton et al.[13] Initially, the original questionnaire was translated into Polish by 2 independent translators (a TMD specialist (PT1) and an English lecturer (PT2)), both fluent in English and native Polish speakers. Subsequently, a consensus was reached to create an agreed version (PT12). Next, a back-translation from Polish to English was conducted by 2 individuals (BT1, BT2), who were native English speakers and unaware of the original English version of the questionnaire.

Once the translations had been verified, the final version was established. To ensure medical accuracy, a committee of specialists, including a TMD specialist and a general dentist, scrutinized the wording. The entire translation process was supervised by a principal investigator who was not directly involved in the translation process. The test received positive evaluations regarding

the clarity of all items, the quality of the language used, its length, and its overall usefulness. Subsequently, the questionnaire was assessed in terms of its ease of completion and comprehensibility within a small sample group (Fig. 1). Accordingly, the final version, which did not require any changes after the prefinal test, was employed in subsequent assessments (Table 1).

Measures

Fonseca Anamnestic Index

The FAI is based on the Helkimo Anamnestic Index,[15] which consists of 10 closed-ended questions. The possible answers to these questions are "yes," "sometimes," or "no," and the answers are assigned values of 10, 5 and 0, respectively. The maximum possible score is 100. A higher score indicates more severe TMD and a greater severity of symptoms. The following division of the total score is proposed: a score of 0–15 is indicative of no dysfunction; a score of 20–40 indicates mild TMD; a score of 45–60 is indicative of moderate TMD; and a score above 60 indicates severe TMD.

RDC/TMD

Two calibrated TMD specialists conducted a clinical examination of the patients. The examination utilized the RDC/TMD questionnaire, which was translated into Polish by Osiewicz et al.[16] This assessment enabled the classification of patients into 3 TMD groups: group I – muscular disorders; group II – disc displacement; group III – arthralgia, osteoarthritis and osteoarthrosis. The study focused on the analysis of Axis I from the questionnaire.

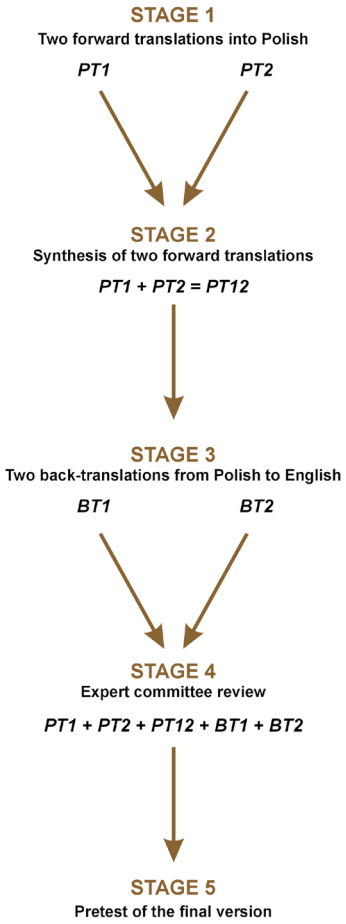

Fig. 1. Stages of the Fonseca Anamnestic Index (FAI) questionnaire translation process into Polish

PT1 – translation of a temporomandibular disorder (TMD) specialist; PT2 – translation of an English lecturer; PT12 – version of the questionnaire based on PT1 and PT2; BT1, BT2 – native English speakers.

Table 1. English and Polish versions of the Fonseca Anamnestic Index (FAI) and the distribution of responses provided by the participants

Question number	English version of the FAI	Polish translation of the questions	Responses		
			no/nie	sometimes/ czasami	yes/tak
Q1	Do you have difficulty opening your mouth wide?	Czy masz trudności z szerokim otwieraniem jamy ustnej?	96 (78.7)	12 (9.8)	14 (11.5)
Q2	Do you have difficulty moving your jaw to the sides?	Czy masz trudności z ruszaniem żuchwą na boki?	99 (81.1)	13 (10.7)	10 (8.2)
Q3	Do you feel fatigue or muscle pain when you chew?	Czy czujesz zmęczeniowy/mięśniowy ból żucia?	66 (54.1)	23 (18.9)	33 (27.0)
Q4	Do you have frequent headaches?	Czy masz częste bóle głowy?	70 (57.4)	19 (15.6)	33 (27.0)
Q5	Do you have neck pain or stiff neck?	Czy występuje u Ciebie ból szyi lub sztywność szyi?	51 (41.8)	41 (33.6)	30 (24.6)
Q6	Do you have earaches or pain in your temporomandibular joint?	Czy występują u Ciebie bóle uszu lub okolicy stawu skroniowo-żuchwowego?	88 (72.1)	14 (11.5)	20 (16.4)
Q7	Have you ever noticed any noise in your temporomandibular joint while chewing or opening your mouth?	Czy kiedykolwiek zauważyłeś jakiekolwiek odgłosy w stawie skroniowo-żuchwowym podczas żucia lub otwierania jamy ustnej?	72 (59.1)	17 (13.9)	33 (27.0)
Q8	Do you have any habits such as clenching or grinding your teeth?	Czy masz jakieś nawyki, takie jak zaciskanie lub zgrzytanie zębami?	30 (24.6)	16 (13.1)	76 (62.3)
Q9	Do you feel that your teeth do not come together well?	Czy czujesz, że Twoje zęby nie kontaktują się w prawidłowy sposób?	80 (65.6)	13 (10.7)	29 (23.8)
Q10	Do you consider yourself a tense (nervous) person?	Czy uważasz siebie za napiętą (nerwową) osobę?	40 (32.8)	33 (27.0)	49 (40.2)

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

Reliability and validity

The reliability of the FAI-PL questionnaire was evaluated through an examination of its internal consistency, as measured by the Cronbach's alpha coefficient, as well as through the application of the test-retest reliability approach. The internal consistency was considered acceptable when the coefficient value exceeded 0.70. The test-retest reliability was evaluated by means of intraclass correlation coefficients (ICCs), using data from the 30 subjects who retook the FAI-PL after a one-week interval.

Construct validity

The construct validity of the FAI-PL was established through an exploratory factor analysis (EFA). Before conducting the EFA, the adequacy of the data was evaluated using the Kaiser–Meyer–Olkin (KMO) test and Bartlett's test of sphericity. These tests were utilized to assess whether the data was suitable for the EFA. It was assumed that a KMO measure below 0.5 would be a clear signal to stop the EFA. To ensure the strength of the factor loadings, each item was required to have a value of ≥0.40 in order to be included in the final selected factor.

Criterion validity

The criterion validity was evaluated, followed by an assessment of the sensitivity and specificity of the FAI-PL in comparison to the RDC/TMD. The sensitivity of the FAI-PL, which represents the ability to identify true positives (i.e., the proportion of TMD individuals correctly identified by the FAI-PL out of the total number of patients with TMD diagnosed by the RDC/TMD), was calculated using the following formula (Equation 1):

$$\text{sensitivity} = \text{true positive}/(\text{true positive} + \text{false negative}) \quad (1)$$

Specificity, which represents the ability to identify true negatives (i.e., the proportion of TMD-free individuals correctly identified by the FAI-PL out of the total number of non-TMD controls established by the RDC/TMD), was calculated using the following formula (Equation 2):

$$\text{specificity} = \text{true negative}/(\text{true negative} + \text{false positive}) \quad (2)$$

Additionally, positive and negative predictive values were calculated. The positive predictive value (PPV) indicates the percentage of individuals with a positive test outcome who have TMD. It can be calculated using the following formula (Equation 3):

$$\text{PPV} = \text{true positive}/(\text{true positive} + \text{false positive}) \quad (3)$$

The negative predictive value (NPV) is the probability that subjects with a negative screening test truly do not have TMD. It is calculated as follows (Equation 4):

$$\text{NPV} = \text{true negative}/(\text{true negative} + \text{false negative}) \quad (4)$$

Statistical analysis

The statistical calculations were conducted using the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA). Descriptive statistics were used to determine the mean values, standard deviation (*SD*), and minimum and maximum values of the demographic variables. The normality of the data distribution was evaluated using the Kolmogorov–Smirnov test. To assess the differences between independent groups, both the *t*-test and the Mann–Whitney *U* test were employed. In all tests, a *p*-value of less than 0.05 was considered statistically significant.

Results

A total of 122 subjects were recruited for this study. Among all participants, 63.9% were female. The mean age of the patients was 28.1 years (*SD*: 6.3).

Table 1 presents the distribution of responses to individual queries in the FAI-PL questionnaire. Notably, patients most frequently reported teeth grinding and clenching, with a frequency of 75.4% (combining "yes" and "sometimes" responses). Conversely, the least frequently reported symptom was difficulty moving the jaw to the sides, noted as 18.9% of positive answers.

The results of the FAI assessment indicated that 27.0% of patients had no TMD symptoms, 35.3% demonstrated mild TMD symptoms, 27.0% displayed moderate TMD symptoms, and 10.7% exhibited severe TMD symptoms. According to the clinical examination based on the RDC/TMD questionnaire, 40.8% of participants had no TMD, 36.7% had myogenous disorders (group I RDC/TMD), 7.5% had joint disorders (group II and group III RDC/TMD), and 16.7% had both. These results are presented in Table 2.

Translation

The FAI-PL has been translated with the utmost fidelity to the original. None of the questions in the questionnaire caused major problems in translation.

Reliability

The Cronbach's alpha coefficient of the FAI-PL was 0.75, which indicates satisfactory internal consistency. The corrected item–total correlations, presented in Table 3, ranged from 0.22 (Q9) to 0.59 (Q3).

Table 2. Frequency of TMD symptoms based on the Polish version of the FAI (FAI-PL) and the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD)

Questionnaire	Result	Frequency *n* (%)
FAI-PL	no TMD	33 (27.0)
	mild TMD	43 (35.3)
	moderate TMD	33 (27.0)
	severe TMD	13 (10.7)
RDC/TMD	no TMD	49 (40.2)
	myogenous disorders	44 (36.1)
	joint disorders	9 (7.4)
	mixed TMD	20 (16.4)

TMD – temporomandibular disorders.

Table 3. Internal consistency and test-retest reliability of the FAI-PL

Question number	Corrected item–total correlation	Cronbach's alpha if item deleted	ICC
Q1	0.40	0.73	0.91
Q2	0.29	0.74	0.84
Q3	0.59	0.69	0.93
Q4	0.47	0.71	0.89
Q5	0.31	0.74	0.87
Q6	0.49	0.71	0.95
Q7	0.38	0.73	0.94
Q8	0.43	0.72	0.91
Q9	0.22	0.75	0.79
Q10	0.49	0.71	0.93

ICC – intraclass correlation coefficient.

All items met the recommended minimum correlation of 0.20 (Table 3). No items should be excluded from the scale in order to improve the Cronbach's alpha. The ICCs for the individual items varied from 0.79 to 0.95 and were mostly excellent. These results suggest that the FAI-PL demonstrates high reliability.

Construct validity

The construct validity was determined by the EFA. The KMO test yielded a value of 0.693, while the result of Bartlett's test was 243.730 (degrees of freedom (*df*) = 45, $p < 0.001$). Subsequently, the number of factors for which the value of the statistic, referred to as the eigenvalue, would exceed 1 (Kaiser's criterion) was determined. As evidenced in Table 4, the data yielded 3 factors and 3 bundles of strongly correlated questions. Those factors explained 55.2% of the total variance observed. Moreover, the factor loadings for all items exceeded 0.40.

The 1st factor consisted of 4 items (Q6, Q7, Q8, Q9), while the 2nd and 3rd factors consisted of 3 items each (Q1, Q2, Q3; and Q4, Q5, Q10, respectively).

Table 4. Factor analysis results for the FAI-PL

Item	Factor 1	Factor 2	Factor 3
Q6 (TMJ pain)	0.602	–	–
Q7 (TMJ sounds during chewing)	0.677	–	–
Q8 (teeth clenching/grinding)	0.665	–	–
Q9 (poor occlusion or bite)	0.580	–	–
Q1 (mouth opening difficulty)	–	0.807	–
Q2 (jaw movement difficulty)	–	0.727	–
Q3 (jaw fatigue or muscle pain)	–	0.534	–
Q4 (frequent headaches)	–	–	0.620
Q5 (neck pain or stiffness)	–	–	0.904
Q10 (emotional stress)	–	–	0.452
Total initial eigenvalue	3.144	1.295	1.079
Rotation sums of squared loadings – percentage variance	21.441	18.168	15.578

TMJ – temporomandibular joint.

Criterion validity

The strength of the agreement between the RDC/TMD and the FAI-PL was moderate, as determined by Cohen's kappa value of 0.68. The diagnostic sensitivity, defined as the ability of the FAI-PL to detect patients with TMD, was 98.6%. In contrast, the diagnostic specificity, defined as the ability of the FAI-PL to exclude the TMD correctly, was 65.3%. At the same time, the PPV, meaning that the subject had TMD with a positive FAI-PL test result, was 80.9%. Conversely, the NPV was 96.9%, suggesting that a negative test result was highly predictive of the absence of TMD.

Discussion

An accurate and comprehensive assessment of TMD is essential for the diagnosis, treatment planning and evaluation of treatment outcomes. In addition to clinical examination and imaging techniques, questionnaires have emerged as valuable tools for gathering patient-reported information, enabling a more holistic understanding of TMD manifestations.

The aim of this research was to translate the FAI questionnaire into Polish and evaluate its psychometric properties. To date, such an attempt has been made by Glowacki et al.[17] However, the study was limited to a cohort of 72 women and lacked a clinical assessment of actual TMD occurrences.[17] To the authors' knowledge, this is the first comprehensive study validating the FAI in a Polish patient population.

The initial phase of the validation process entailed the translation of the FAI into Polish, which represented a pivotal step in the process of questionnaire validation. The translation process aimed to maintain fidelity to the original English version. A subsequent back-translation revealed no notable conceptual deviations from the source material.

With regard to the reliability of the FAI-PL, the Cronbach's alpha coefficient was found to be 0.75. The Indonesian version of the questionnaire demonstrated an alpha statistic score of 0.57,[18] while the Chinese version by Zhang et al. yielded the score of 0.67.[19] The Malay questionnaire achieved a Cronbach's alpha of 0.90, and the Turkish version displayed a notably high value of 0.95.[20,21] Consequently, the outcome for the Polish version falls relatively midway when compared to other adaptations, resembling the alpha score reported by Alyessary et al. for the Arabic version (0.77).[11] It is vital to underscore that a reliability threshold of at least 0.7 is typically regarded as dependable. The test-retest reliability varied from 0.79 to 0.95 for individual items, with the majority of results falling within the excellent range, which is consistent with the findings of other studies. According to Yap et al., this is likely attributable to the brevity and simplicity of the FAI.[20]

The EFA revealed a three-factor structure of the FAI-PL. Each of the 3 factors exhibited eigenvalues greater than 1. Principal component analysis demonstrated that these 3 factors explained a satisfactory proportion of the overall variance. The 1st factor includes questions related to the TMJ (TMJ pain, TMJ sounds) and interdental interactions (grinding/clenching, poor occlusion). The 2nd factor is related to jaw mobility (difficulty with jaw movement, mouth opening and jaw fatigue). The 3rd factor covers the remaining questions not directly related to the TMJ and masticatory muscles (including neck pain, headaches and stress). The three-dimensional structure of the FAI has been corroborated in other studies.[11,22,23] Nonetheless, in each of these studies, different questions were incorporated within the respective factors. The second factor derived from our study (Q1, Q2, Q3) aligns with a factor identified by Alyessary et al. as parafunction-related.[11] Similarly, the third factor (Q4, Q5, Q10) corresponds with the second dimension outlined in the study by Rodrigues-Bigaton et al.[22] It is noteworthy that the factor analysis conducted by Rodrigues-Bigaton et al. served as the foundation for the development of the Short-Form Fonseca Anamnestic Index (SFAI).[24] The SFAI comprises 5 questions extracted from the original FAI questionnaire (Q1, Q2, Q3, Q6, Q7). In contrast, the investigation by Arikan et al. revealed a two-factor structure for the Turkish version of the FAI.[21] These factors were categorized as function-comorbidity-related (Q1–Q7) and occlusion-parafunction-psychology-related (Q8–Q10).

Considering the criterion validity, the FAI-PL shows a high degree of agreement with the RDC/TMD Axis I diagnoses. In previous studies, the FAI sensitivity rates ranged from 83.3% to 97.2%.[18,19,23,25] In our research, a very high sensitivity rate of 98.6% was achieved. Although the sensitivity was remarkably high, the specificity of the test was considerably lower (65.3%). The results were consistent with those reported by other authors.[19,23,25] The studies indicate that the FAI demonstrated high sensitivity in identifying individuals with TMD. However, the test's specificity in distinguishing individuals without TMD in relation to the RDC/TMD or DC/TMD was limited. As Yap et al. have observed, the FAI questionnaire includes a number of questions that are not specific to TMD (neck pain, headache, stress).[20] In conclusion of these findings, the FAI can serve as a preliminary tool for evaluating individuals with TMD symptoms and classifying the condition. However, a thorough clinical assessment is essential to ensure an accurate diagnosis following the use of the FAI.[25–27]

It is worth noting that the FAI is not the only questionnaire used to screen patients with TMD. Another instrument documented in the literature is the TMD Pain Screener (TPS), which is incorporated into the DC/TMD and the 3 screening questions (3Q/TMD).[28,29] The TPS comprises questions specifically addressing pain, while the 3Q/TMD explores both the occurrence of pain and intra-articular disorders. In screening tests, the latter option is becoming increasingly prevalent.[30,31]

Limitations

The findings of the present study are limited by a number of factors. Firstly, it should be noted that the Polish translation was derived from the English version, rather than the original Portuguese text. This may potentially impact the final Polish version of the questionnaire. Secondly, the responsiveness of the FAI-PL was not examined. Further studies are required to assess the impact of the applied treatment on the FAI-PL outcomes. In the present study, the RDC/TMD was employed as the gold standard for diagnosing TMD, given the absence of a validated Polish version of the DC/TMD at the time of article creation.

Conclusions

In summary, the Polish adaptation of the FAI serves as a valuable tool in the diagnosis of TMD, as it effectively captures patient-reported symptoms, functional constraints and psychosocial aspects. Nevertheless, it is essential to use the FAI-PL in conjunction with clinical assessments and imaging procedures to ensure a thorough and precise diagnosis. This assessment demonstrates strong internal consistency, repeatability, and sound construct and criterion validity. In light of these findings, it can be concluded that the FAI-PL is a reliable instrument for use in both clinical settings and research within the Polish context.

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee at Poznan University of Medical Sciences, Poland (protocol No. 522/21). Prior to their participation in the study, all participants were duly informed and provided written consent.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Małgorzata Gałczyńska-Rusin https://orcid.org/0000-0003-2264-6577
Małgorzata Pobudek-Radzikowska https://orcid.org/0000-0002-0828-5771
Agata Czajka-Jakubowska https://orcid.org/0000-0002-1692-2910

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Original paper

Avelumab reduces STAT3 expression with effects on IL-17RA and CD15

Wojciech Kacper Szlasa[1,A], Natalia Julia Sauer[2,B], Jakub Karwacki[3,C], Anna Szewczyk[3,C,D], Julita Kulbacka[3,E], Tomasz Szydełko[1,E], Jolanta Saczko[3,F], Bartosz Malkiewicz[1,A,B,F]

[1] Department of Minimally Invasive and Robotic Urology, University Center of Excellence in Urology, Wroclaw Medical University, Poland
[2] Department of Drug Form Technology, Faculty of Pharmacy, Wroclaw Medical University, Poland
[3] Department of Molecular and Cellular Biology, Faculty of Pharmacy, Wroclaw Medical University, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):713–720

Address for correspondence
Wojciech Kacper Szlasa
E-mail: wojtek.szlasa@outlook.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on July 15, 2023
Reviewed on November 22, 2023
Accepted on December 5, 2023

Published online on October 31, 2024

Abstract

Background. Avelumab is a human antibody that targets the programmed cell death ligand-1 (PD-L1) protein in cancer cells. Novel anticancer therapies for renal cell carcinoma (RCC) consider cluster of differentiation 15 (CD15) and interleukin 17 receptor A (IL-17RA) as potential targets. Notably, the expression of PD-L1, CD15 and IL-17RA is dependent on signal transducer and activator of transcription 3 (STAT3).

Objectives. The aim of the study was to investigate whether targeting PD-L1 with avelumab alters the expression levels of CD15 and IL-17RA, and to assess the STAT3-mediated regulation of CD15 and IL-17RA.

Material and methods. We applied immunocytochemistry (ICC) and confocal laser scanning (CLS) microscopy to assess the expression and localization of the immunotherapy targets in 3 renal cancer cell lines and 1 healthy renal cell line.

Results. After treatment with 20 ng/mL avelumab, renal cancer cells showed a reduction in STAT3 expression. The expression of CD15 increased in cancer cells that exhibited a high level of IL-17RA, and the membrane signal of CD15 was reduced. In other renal cancer cell lines, the expression of CD15 decreased. Conversely, the level of IL-17RA changed only in healthy renal cells after treatment with avelumab, with no impact on renal cancer cells.

Conclusions. Our study suggests that the targeting of PD-L1 with avelumab alters the expression of CD15 and IL-17RA, which play an important prognostic and therapeutic role in novel anticancer therapy.

Keywords: CD15, avelumab, IL-17RA, membrane antigen modulation

Cite as
Szlasa WK, Sauer NJ, Karwacki J, et al. Avelumab reduces STAT3 expression with effects on IL-17RA and CD15. *Dent Med Probl.* 2024;61(5):713–720. doi:10.17219/dmp/176374

DOI
10.17219/dmp/176374

Introduction

Renal cell carcinoma (RCC) is a type of kidney cancer that originates in the lining of small tubes within the kidney.[1] There are several subtypes of RCC, including clear cell RCC, papillary RCC and chromophobe RCC. Clear cell RCC is the most common subtype, accounting for 75% of all RCC cases.[1,2] Renal cell carcinoma is among the most lethal urological cancers due to its delayed diagnosis and poor response to therapies. Surgery is the primary treatment for localized RCC, with partial or radical nephrectomy being performed depending on the size and location of the tumor.[1] For advanced RCC, systemic therapy with targeted agents such as sunitinib, pazopanib and axitinib is the standard of care.[1,2] Immunotherapy with nivolumab, ipilimumab and avelumab is also used in the treatment of advanced RCC.[1,2]

Avelumab is a human monoclonal immunoglobulin G1 (IgG1) antibody that targets the programmed cell death ligand-1 (PD-L1) protein, which is expressed in cancer cells. It has been approved as a monotherapy for the treatment of patients with Merkel cell carcinoma and urothelial carcinoma. Avelumab is used in combination with axitinib for the treatment of RCC.

By acting on PD-L1, avelumab interferes with various downstream signaling pathways, affecting the expression of various proteins. Among the potential membrane antigens which are affected by anti-PD-L1 therapy, cluster of differentiation 15 (CD15) and interleukin 17 receptor A (IL-17RA) have been identified as novel targets for the anticancer therapy of various tumors and diseases.[1,3]

The expression of CD15 and CD15s is linked to lymphatic and venous invasion, lymph node metastasis, distant metastasis, tumor stage, tumor recurrence, and overall survival in cancer.[4] These antigens have the potential to serve as biomarkers for the diagnosis and prognosis in various types of cancer and may represent therapeutic targets.[5] However, further research into the complex expression of CD15 and CD15s is needed to properly classify diagnostic parameters. The CD15 has demonstrated efficacy as a target for cancer therapy, affecting both cancer cells and immune myeloid-derived cells. This leads to an improved response to therapy as well as the inhibition of cancer growth and progression in tumor microenvironments.[6] Experimental and clinical studies have demonstrated that targeting CD15 and CD15s is a promising treatment approach.[7] However, ongoing efforts should focus on refining the clinical application of anti-CD15 therapy and assessing its safety profile.

Renal cell carcinoma is closely associated with immune mediators such as IL-17, which is an inflammatory cytokine that responds to tissue damage and external pathogens. The antigen IL-17RA is an interleukin receptor that is expressed in various kidney cells, including podocytes, mesangial cells and renal proximal tubular endothelial cells.[8–10] The relationship between IL-17 and tumors is complex and has become an area of interest for numerous studies. Interleukin-17 plays a dual role in cancer development, as it can be both pro- and anti-tumorigenic.[8] The interaction between IL-17 and IL-17RA has been shown to promote neoplasm metastasis and induce angiogenesis.[11] A study by Dębiński et al. demonstrated the significance of lymphangiogenesis, especially intratumoral lymphatics, in aggressive cases of RCC.[12] Additionally, IL-17 signaling is thought to similarly affect the tumor microenvironment.[13]

Escors et al. have previously described the effects of the PD-L1 intracellular signalosome in cancer cells.[14] The cancer cells alter the protein expression via signal transducer and activator of transcription 3 (STAT3) phosphorylation.[14] The effects of IL-17A in kidney epithelial cells are exerted via the ERK1/2 and STAT3 pathways.[10,15] The expression of CD15 has been observed to inversely correlate with STAT3 levels.[16] When the level of CD15 increases, the level of STAT3 decreases. Therefore, the expression levels of both CD15 and IL-17RA depend on the expression of STAT3 and its phosphorylation level.

Avelumab, a PD-L1-targeting antibody, has shown efficacy in the treatment of various cancers. However, its impact on critical antigens such as CD15 and IL-17RA remains unexplored. The CD15, increasingly recognized in RCC prognosis, has an influence on invasion, metastasis and overall survival. Its potential as a diagnostic and therapeutic marker emphasises the need for comprehensive investigation. Concurrently, IL-17RA, which is expressed in renal cells, plays a dual role in cancer, influencing both metastasis and angiogenesis. The relationship between PD-L1, CD15, IL-17RA, and their downstream signaling pathways remains understudied. This research aims to address this gap in knowledge by investigating the effects of avelumab on the expression of CD15 and IL-17RA. A comprehensive understanding of the manner in which avelumab modulates these antigens is pivotal for advancing personalized therapies, unraveling novel biomarkers and enhancing our understanding of cancer antigen modulation by anticancer drugs. This short study focuses on the changes in CD15 and IL-17RA expression after treatment of the cells with avelumab, addressing a crucial knowledge gap and shedding light on the emerging role of cancer antigen modulation by anticancer drugs.[17] Moreover, it establishes a foundation for more refined therapeutic strategies and improved patient outcomes.

Material and methods

Primary cell culture

Renal cancer cells (RC1, RC2, RC3) and healthy renal cells (HR) were derived from the renal cancer resections obtained from patients at the Department of Minimally Invasive and Robotic Urology (University Center of

Excellence in Urology, Wroclaw Medical University, Poland). The corresponding part of the resected tissues underwent pathological examination. The RC1 cells were derived from a 68-year-old female patient with G1 clear cell RCC (primary tumor subtype 1a (pT1b)). The RC2 cells were derived from a 69-year-old male patient with G2 clear cell RCC (pT1b). The RC3 cells were derived from a 78-year-old female patient with G2 clear cell RCC (pT1b). The HR cells were derived from a 59-year-old male patient with G2 papillary cell RCC (pT1b). The use of patient-derived cells from the same individual provides a more clinically relevant and representative model for studying RCC and allows researchers to better understand the specific characteristics of both cancerous and healthy cells within the context of an individual's unique biological profile. After the surgical removal of the tumor, 0.5 cm^3 of cancer tissue was inserted into the 2-mL probe with phosphate-buffered saline (PBS; Sigma-Aldrich, St. Louis, USA). The sample was immediately transported to the laboratory and further cut into small pieces. The tissue samples were incubated in a culture medium, which was replaced daily. The cells were cultured in 25-mL polystyrene cell culture flasks (Falcon®; Corning Life Sciences, Tewksbury, USA) with Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich) and antibiotic (streptomycin/gentamycin) in a humidified incubator (Heracell; Thermo Fisher Scientific, Waltham, USA) under standard culture conditions at 37°C in an atmosphere containing 5% CO_2. When required, the cells were rinsed with PBS and removed by trypsinization (0.025% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA); Sigma-Aldrich). The cells were resuspended and transferred into new culture flasks. The flasks in which the cells exhibited consistent morphology were trypsinized, and a small number of cells were transported to the new culture flask.

Avelumab preparation and incubation

The experimental protocol involved seeding the renal cancer cells on glass coverslips and 10-well microscopy slides to facilitate subsequent analyses. Avelumab, sourced as Bavencio in a 20 mg/mL concentration, was prepared by dissolution in DMEM to attain concentrations of 20 ng/mL and 20 µg/mL. Subsequently, the requisite quantity of the drug solution was administered to the cells, initiating a 24-hour incubation period. Following this exposure, the cells were fixed using 4% paraformaldehyde. Then, immunocytochemical and fluorescent staining procedures were employed to visualize and assess the expression patterns of CD15 and IL-17RA for potential alterations induced by avelumab at the molecular level. This methodological approach aimed to capture and analyze the responses of RC cells to varying concentrations of avelumab, thereby laying a foundation for understanding its impact on CD15 and IL-17RA expression.

MTT assay

In this study, the assessment of cell viability and metabolic activity was conducted using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. After cultivating renal cancer cells (RC1, RC2, RC3) and HR cells under specified culture conditions in polystyrene cell culture flasks, the MTT assay was employed to assess cellular health. Following the seeding of cells in 96-well plates, the cells were treated with the MTT reagent and incubated, allowing viable cells to enzymatically convert the dye into formazan crystals. Subsequently, the formazan crystals were solubilized, and a spectrophotometric measurement was conducted at approx. 570 nm, providing a quantitative readout of cell viability. The colorimetric assay served as a crucial tool for evaluating the impact of experimental variables on the metabolic activity of the renal cancer cells, offering insights into their proliferative and survival capacities. The MTT assay, a standard method in cell biology, facilitated a robust assessment of cellular responses and contributed valuable data to the characterization of RC and HR cells.

Immunocytochemical staining

The immunocytochemical staining was employed to semi-quantitatively assess the expression and distribution of CD15, IL-17RA and STAT3 in RC1–RC3 and HR cell lines following the incubation with avelumab. The cells were incubated for 24 h on 10-well microscopy slides, washed in PBS, and fixed in 4% formalin. Afterward, they were washed in PBS, and a peroxide block (ab80436 kit; Abcam, Waltham, USA) was added for 10 min. Subsequently, the cells were washed in PBS with 1% Triton X (Merck Life Science Sp. z o.o., Poznań, Poland). The protein block agent (Abcam) was incubated with the cells for 10 min. The cells were then incubated with the first-order antibodies (1:250, STAT3 (124H6); 1:250, IL-17R (49M4D2); 1:250, CD15 (sc-19648)) for 24 h at 4°C. Afterwards, the cells were washed with PBS and 1% Triton X, and the mouse complement agent (Abcam) was added. After 10 min, the cells were washed with PBS and 1% Triton X. Then, a 3,3'-diaminobenzidine (DAB) mixture (1 DAB:50 DAB substrate) was applied for 10 min in the dark. Next, the cells were washed for 10 min in distilled water and stained with hematoxylin for 1 min. The excess stain was removed by washing with water for 30 min, and the cells were dehydrated by incubation for 5 min in each of the ethanol solutions (50%, 60%, 70%, 80%, 90%, and 96%). In the final stage of sample preparation, xylene I and II were used to dehydrate the sample. Finally, the glass was mounted on the sample using dibutylphthalate polystyrene xylene (DPX) medium (Aqua-Med ZPAM-KOLASA, Łódź, Poland). The samples were observed under a light microscope (Olympus BCX43; Olympus, Tokyo, Japan) and the images were captured using a Plan-Apochromat 20× objective (Olympus). In each sample, the percentage of stained cells was recorded.

The immunoreactive score (IRS), developed by Remmele and Stegner, is a widely utilized method for the semi-quantitative assessment of immunohistochemical staining in histopathology.[18] Introduced in 1987, the IRS integrates both the intensity and proportion of stained cells to provide a comprehensive score reflecting the overall immunoreactivity within a tissue sample. The IRS system assigns a numeric value to the staining intensity (ranging from 0 to 3) and another value to the proportion of positively stained cells (ranging from 0 to 4). These values are then multiplied, resulting in a final IRS ranging from 0 to 12. This scoring system allows researchers and pathologists to objectively evaluate the expression levels of specific antigens in tissues, thereby contributing to the understanding of disease processes and aiding in the development of targeted therapeutic approaches. The IRS has been particularly valuable in the field of cancer research, where the assessment of the extent and intensity of protein expression can provide insights into tumor biology and prognosis.

Fluorescent staining

To visualize and assess the expression ratio of STAT3 to phospho-STAT3 and IL-17RA to IL-17, confocal laser scanning (CLS) microscopy was used. The cells were incubated on covered glass slides within Petri dishes after 24 h and washed 3 times with PBS. The fixed cells were labeled with the first-order antibodies (1:250, STAT3 (124H6; Cell Signaling Technology, Inc., Danvers, USA); 1:250, IL-17R (49M4D2; Novus Biologicals, Centennial, USA); 1:250, CD15 (sc-19648; Santa Cruz Biotechnology, Dallas, USA)) for 1 h at 37°C, in accordance with the manufacturer's

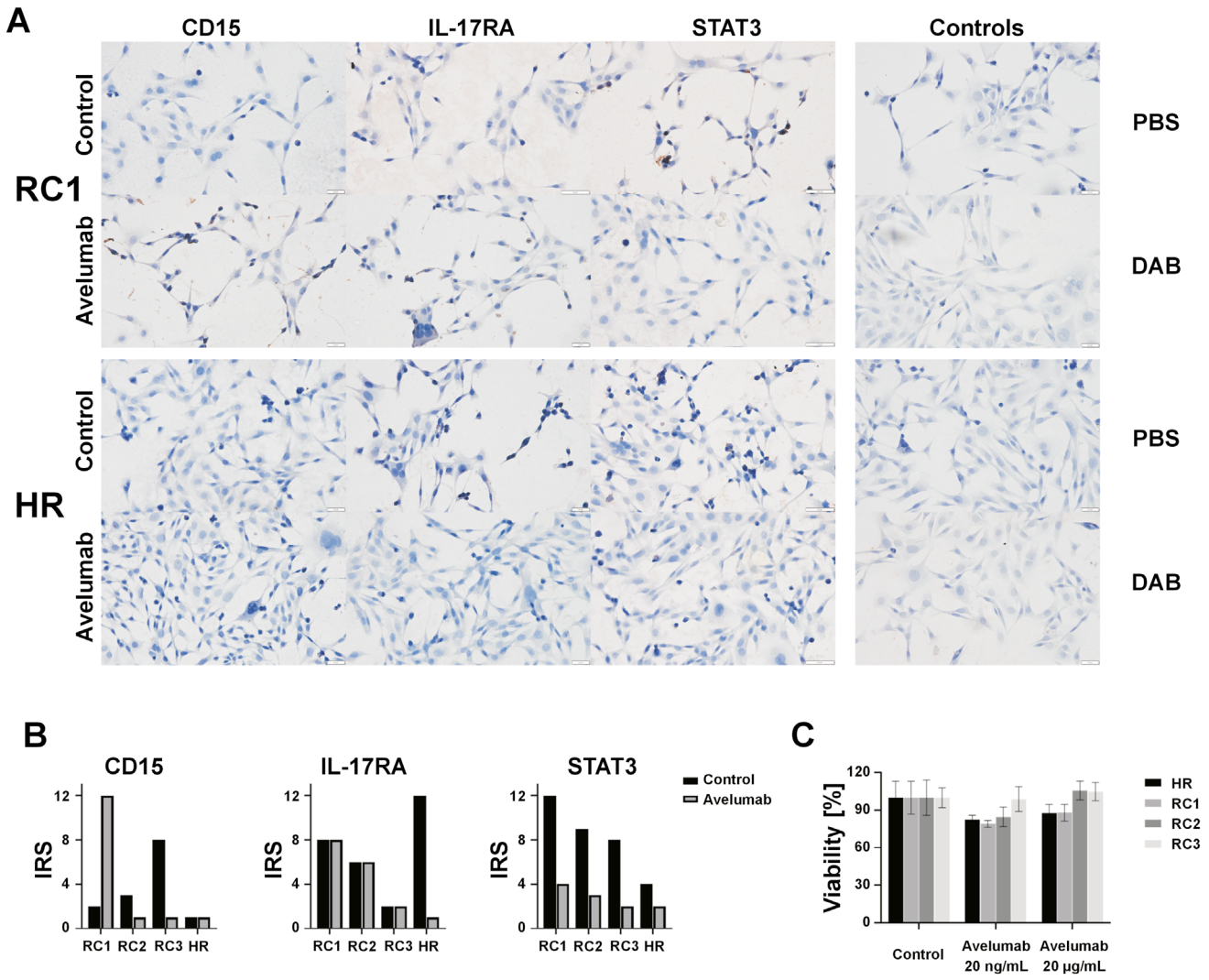

Fig. 1. Effects of avelumab on the expression of cluster of differentiation 15 (CD15), interleukin 17 receptor A (IL-17RA) and signal transducer and activator of transcription 3 (STAT3) in renal cancer cells (RC1–RC3) and healthy renal cells (HR)

A. Immunocytochemistry (ICC) reaction showing the expression of CD15, IL-17RA and STAT3 in RC1 and HR with control immunohistochemistry (ICH) samples; B. Effects of 20 ng/mL avelumab on the expression of CD15, IL-17RA and STAT3 in 3 renal cancer cell lines and HR examined by ICC and assessed using the immunoreactive score (IRS) pathology classification; C. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay following a 24-h incubation period with avelumab at concentrations of 20 ng/mL and 20 µg/mL
PBS – phosphate-buffered saline; DAB – 3,3'-diaminobenzidine.

protocol. Next, the cells were washed with PBS and stained with the second-order antibodies (Alexa Fluor™ 594 and Alexa Fluor™ 488 (1:500; A32731 and A32744, respectively; Abcam) for 1 h at 37°C. Fluoroshield™ with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma-Aldrich) was applied for the visualization of nuclei and for mounting the cells. The cells were observed using the Olympus FluoView FV1000 CLS microscope (Olympus) and the images were captured using a Plan-Apochromat (60×) oil immersion objective (Olympus).

Statistical analysis

The viability and microscopy experiments were performed in at least 3 replicates. The data was expressed as mean ± standard deviation (*M* ±*SD*) and analyzed using one-way analysis of variance (ANOVA) (GraphPad Prism 8; GraphPad Software, Boston, USA). A p-value <0.05 was considered statistically significant. The immunocytochemical reaction was performed for each sample in triplicate.

Results

The results of our study demonstrate a profound and selective downregulation of STAT3 expression in renal cancer cells in response to avelumab, as illustrated in Fig. 1A. This downregulation was specifically associated with both the total STAT3 protein and its phosphorylated form, as depicted in Fig. 2A and Fig. 2B.

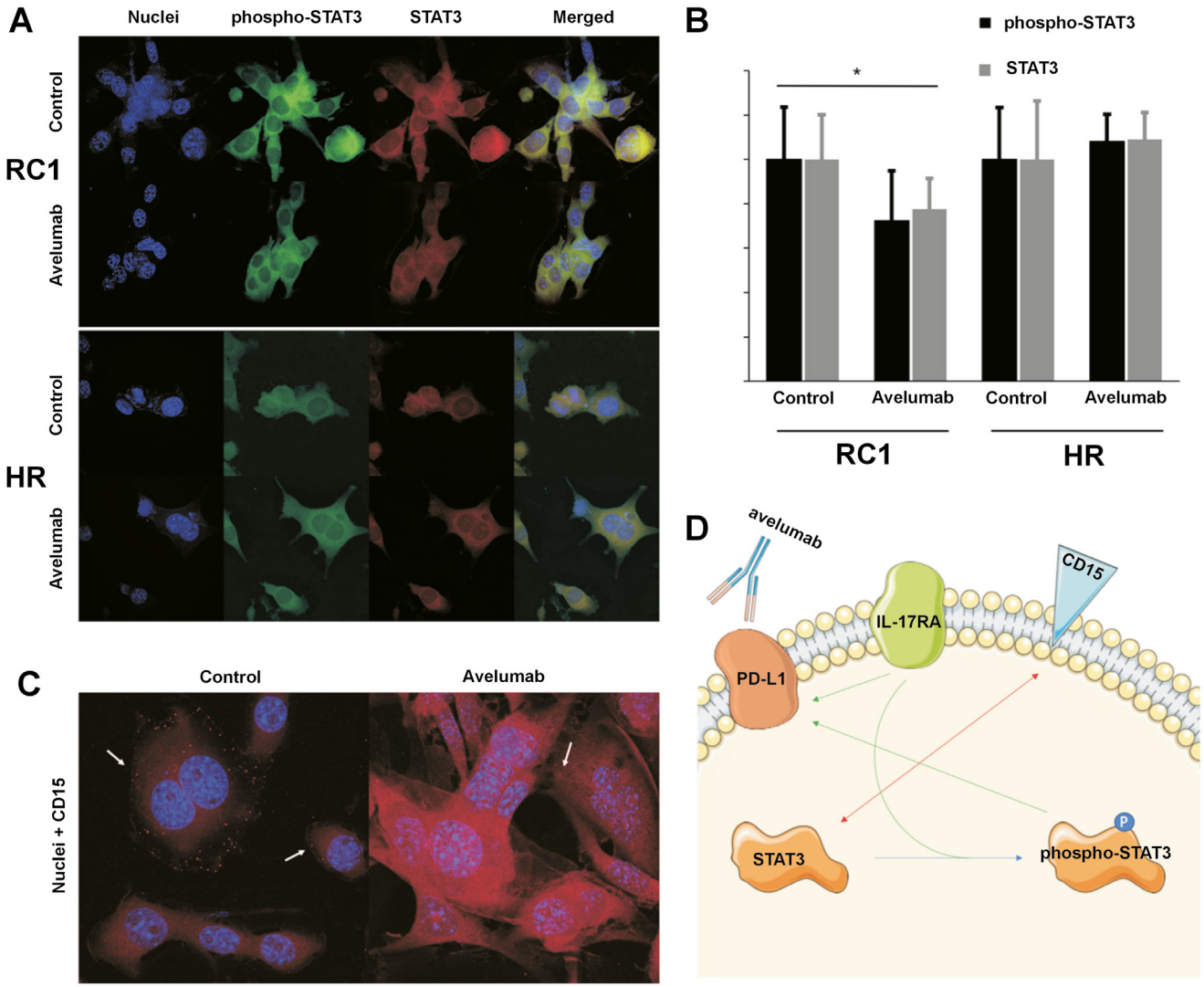

Fig. 2. Confocal laser scanning (CLS) microscopy photographs following the incubation with avelumab, and the interactions between STAT3, IL-17RA and CD15

A. CLS microscopy studies of STAT3 and phospho-STAT3 following the incubation with avelumab; B. Fluorescence analysis of the depicted photographs; C. CLS microscopy photographs of CD15 expression in RC1 cells following the incubation with 20 ng/mL avelumab. White arrows depict the membrane localization of CD15; D. Scheme showing the interactions between STAT3, IL-17RA and CD15. Programmed cell death ligand-1 (PD-L1) expression is upregulated by signaling from IL-17RA. Phospho-STAT3 binds directly to the PD-L1 promoter and increases its expression. STAT3 and CD15 are expressed in different differentiation stages of the cells and thus, only one of the antigens is expressed on the cancer cell membrane. The antibody regulates the expression of CD15, IL-17RA and STAT3 via binding of avelumab to PD-L1

* statistically significant ($p < 0.05$, one-way analysis of variance (ANOVA)).

The HR cells did not exhibit a similar response to avelumab, indicating that the observed effect was specific to cancerous cells. Furthermore, despite the marked changes in STAT3 expression, no discernible morphological alterations were observed in the renal cancer cells following the incubation with avelumab.

Our investigations using immunocytochemistry (ICC) have revealed an intriguing antagonistic relationship between CD15 and IL-17RA expression, as illustrated in Fig. 1B. Specifically, when the expression of CD15 was influenced by avelumab treatment, there was no concomitant change in the expression of IL-17RA. Upon exposure to avelumab, the RC1 cell line exhibited a shift of CD15 from the cell membrane to the membranous organelles inside the cells, as depicted with white arrows in Fig. 2C. Despite the shift in the localization of CD15, the overall expression of CD15, as observed in both ICC and immunofluorescent studies, exhibited an unexpected increase.

Conversely, a decrease in IL-17RA levels in the HR cells following avelumab incubation was not accompanied by a corresponding change in the expression of the receptor in the renal cancer cells. This intriguing finding suggests that avelumab may exert distinct and cell-specific effects on IL-17RA expression, with HR cells responding differently than their cancerous counterparts. The differential response of renal cancer cells and healthy cells to avelumab underscores the complexity of the drug's impact on signaling pathways and highlights the necessity for further exploration of its precise mechanisms of action.

The observed alterations do not affect the viability of the cells, as evidenced by the absence of statistically significant results (Fig. 1C). Noteworthy, avelumab, a drug that affects the immune system, is not solely responsible for cytotoxicity in the cancer cell culture. These results contribute to a more comprehensive understanding of the molecular responses induced by avelumab in renal cancer cells. The selective downregulation of STAT3, the antagonistic relationship between the expression of CD15 and IL-17RA, and the differential responses in renal cancer cells and healthy cells provide valuable insights that may have implications for the development of targeted therapies in renal cancer. The absence of morphological changes in cancer cells following avelumab treatment further emphasizes the specificity of the observed molecular alterations. Our findings pave the way for future research into the intricate interplay of signaling pathways affected by avelumab in renal cancer, offering potential avenues for therapeutic interventions and personalized treatment strategies.

Discussion

The STAT3 signaling is responsible for the simultaneous alterations in CD15 and IL-17RA. Several studies have indicated that IL-17RA-mediated signaling leads to the phosphorylation of STAT3, which in turn activates the transcription of PD-L1.[15,19,20] Yang et al. demonstrated that the expression of IL-17RA is associated with the promotion of cancer stem-like properties in cancer cells via the STAT3 pathway.[21] The findings of the study conducted by Hevehan et al. showed that the expression of STAT3 remains strongest in $CD15^-$ cells and gradually declines upon granulocytic differentiation, thereby supporting the negative regulation of protein expression.[22] Giordano et al. demonstrated that STAT1/STAT3-dependent phosphorylation does not cause CD15 overexpression, indicating that the shift from STAT3 to phospho-STAT3 does not independently affect the expression of CD15.[23]

A summary of the interaction between CD15, IL-17RA and STAT3 is shown in Fig. 2D. A study by Zerdes et al. demonstrated that STAT3 promotes PD-L1 expression[24] and another study showed that phospho-STAT3 increases the expression of PD-L1 through direct binding to the PD-L1 promoter.[25] The targeting of IL-17A has been observed to inhibit the expression of PD-L1 in tumor cells.[26,27] Escors et al. discussed the impact of PD-L1 intracellular signalosome in cancer cells, which modifies protein expression through STAT3 phosphorylation.[14] Meanwhile, IL-17A was found to affect kidney epithelial cells through the extracellular signal-regulated kinase 1/2 (ERK1/2) and STAT3 pathway,[10,15] with CD15 expression correlating to STAT3 levels.[16] Interestingly, the levels of both proteins were observed to act in a reverse manner, with STAT3 levels decreasing when CD15 levels increased. Consequently, the expression levels of CD15 and IL-17RA were found to depend on the expression of STAT3 and its level of phosphorylation. There are currently no studies on the impact of avelumab on the expression of CD15 and IL-17RA. To address this gap in knowledge, we aimed to investigate the changes in CD15 and IL-17RA expression following treatment with avelumab, thereby deepening our understanding of the potential role of anticancer drugs in modulating cancer antigens.[17]

The findings of this study offer valuable insights into the effects of avelumab on the expression levels of PD-L1, CD15 and IL-17RA in renal cancer cells. However, further research is required to fully understand the underlying mechanisms and to optimize the use of avelumab and other immunotherapeutic agents in the treatment of RCC. Future studies may explore the impact of combination therapies targeting multiple signaling pathways and proteins involved in RCC, as well as the use of biomarkers to identify patients who are most likely to benefit from immunotherapy. In addition, the study of the effect of avelumab on other cell types, such as tumor-associated immune cells and stromal cells, may provide additional insights into the complex interactions between different cell types within the tumor microenvironment. Ultimately, continued research in this area has the potential to facilitate the development of more effective and personalized therapies for patients with RCC.

Limitations

While our study provides valuable insights into the impact of avelumab on the expression levels of PD-L1, CD15 and IL-17RA in renal cancer cells, it is important to acknowledge certain limitations that warrant consideration. Firstly, the lack of existing studies regarding the influence of avelumab on the expression of CD15 and IL-17RA necessitates a cautious interpretation of our findings. Further investigations are required to validate and expand upon our observations. Additionally, the inherent complexity of the tumor microenvironment introduces a degree of variability, as evidenced by the observed differences among patients (RC1–RC3) in our study. The high degree of variability observed in the results may also be attributed to inherent biological heterogeneity within the patient cohort and potential variations in the tumor microenvironment, genetic makeup and previous treatment histories. Recognizing the significance of this variability, future studies should aim to address and elucidate these patient-specific factors, employing larger cohorts and comprehensive patient profiling to enhance the generalizability and robustness of our findings. Despite these limitations, our study serves as a foundational exploration into the potential effects of avelumab on RCC. It provides a platform for further research and the refinement of therapeutic strategies in the pursuit of more effective and personalized treatment approaches.

Conclusions

In targeting renal cells with avelumab, our study unveils notable alterations in the expression of CD15 and IL-17RA antigens. Significantly, the complex interplay between STAT3 and the phospho-STAT3 pathway emerges as a pivotal mechanism orchestrating these molecular changes. While our findings underscore the potential of these antigens as promising targets in novel anticancer therapies, further investigations involving sophisticated in vivo studies and an expanded research cohort encompassing a greater number of patients in study samples are required to further corroborate our findings. These endeavors will not only serve to fortify the robustness of our results and hypotheses but also contribute to the development of more precise and personalized therapeutic regimens, ultimately advancing the clinical applicability of avelumab in RCC.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Wroclaw Medical University (approval No. 755/2022).

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Wojciech Kacper Szlasa https://orcid.org/0000-0002-5477-5771
Natalia Julia Sauer https://orcid.org/0000-0002-1898-3952
Jakub Karwacki https://orcid.org/0009-0005-4441-1877
Anna Szewczyk https://orcid.org/0000-0003-0934-9180
Julita Kulbacka https://orcid.org/0000-0001-8272-5440
Tomasz Szydełko https://orcid.org/0000-0002-1360-0422
Jolanta Saczko https://orcid.org/0000-0001-5273-5293
Bartosz Malkiewicz https://orcid.org/0000-0002-5933-3753

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Original paper

Novel combination of nanostructured calcium hydroxide and natural materials: Formulation and characterization

Anastasia Elsa Prahasti[1,2,A,B,D,F], Tamara Yuanita[3,A,C,E], Retno Pudji Rahayu[4,B,C,E]

[1] Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia
[2] Department of Conservative Dentistry, Universitas Trisakti, Jakarta, Indonesia
[3] Department of Conservative Dentistry, Airlangga University, Surabaya, Indonesia
[4] Department of Oral and Maxillofacial Pathology, Airlangga University, Surabaya, Indonesia

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):721–728

Address for correspondence
Tamara Yuanita
E-mail: tamara-y@fkg.unair.ac.id

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
The authors would like to thank the Dental Material and Testing Center of Research and Education (Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia), Prodia StemCell Indonesia (ProSTEM), and Prodia Education and Research Institute (PERI) for their invaluable support.

Received on November 16, 2022
Reviewed on February 17, 2023
Accepted on March 8, 2023

Published online on October 24, 2024

Cite as
Prahasti AE, Yuanita T, Rahayu RP. Novel combination of nanostructured calcium hydroxide and natural materials: Formulation and characterization. *Dent Med Probl.* 2024;61(5):721–728. doi:10.17219/dmp/161988

DOI
10.17219/dmp/161988

Abstract

Background. Calcium hydroxide is used as a reparative dentin inducer. The combination of cocoa pod husk and calcium hydroxide has also been studied as a reparative dentin inducer, whereas anchovy was identified as a good calcium source.

Objectives. The aim of the study was to investigate the optimal ratio of a nano-combination of calcium hydroxide, cocoa pod husk extract and anchovy powder as a reparative dentin inducer by analyzing their material, antioxidant and cytotoxic properties.

Material and methods. The ratios of the 3 elements were established and their performance was compared in terms of viscosity, setting time, pH, and solubility rate. The optimal ratio was then examined for its antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Additionally, its cytotoxicity against human dental pulp stem cells (hDPSCs) was evaluated through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results. A ratio of 1:1:1 of calcium hydroxide, cocoa pod husk extract and anchovy powder was identified as the optimal ratio. It exhibited the greatest flowability, the shortest final setting time, had a pH range of 8.5–10.5, and the second highest solubility rate in comparison with other ratios. Moreover, it demonstrated active radical scavenging activity and stimulated the proliferation of hDPSCs.

Conclusions. Based on the material, antioxidant and cytotoxic properties, a ratio of 1:1:1 of nano-calcium hydroxide, cocoa pod husk extract and anchovy powder was determined to be optimal for use as a reparative dentin inducer.

Keywords: good health and well-being, reparative dentin inducer, novel nano-combination

Introduction

Direct pulp capping is a difficult dental procedure that aims to preserve the vitality of the pulp. The selection of cases and an optimal healing environment are required to achieve a favorable outcome. Complications may arise due to this treatment, including damage to odontoblast cells in the predentin layer, pulpal bleeding, bacterial contamination, and biomaterial properties.[1–3] Direct pulp capping is performed to stimulate the formation of hard tissue from the inflamed pulp, thus forming a closure on the perforation in the residual pulp. The success of the treatment depends on the materials used in the procedure.[4] For several decades, calcium hydroxide has been the most commonly implemented material, as it exhibits antimicrobial and mineralizing properties that facilitate pulpal healing. These properties are unparalleled among currently available biomaterials.[5,6] Additionally, calcium hydroxide is preferred due to its favorable pH and anti-inflammatory effect.[4] However, the limitations of calcium hydroxide include the formation of multiple tunnel defects on the induced reparative dentin. In order to address this issue, a number of new biomaterials have been developed.

Mineral trioxide aggregate (MTA) is a biomaterial under consideration as a replacement for calcium hydroxide in the context of reparative dentin induction. Mineral trioxide aggregate can induce reparative dentin with a structure similar to that of primary dentin while causing no pulp inflammation.[7,8] However, MTA is not easy to use, and the acidic environment affects its setting time. In the previous study, MTA showed more cervical discoloration than calcium-enriched mixture cement,[9] which could be considered a disadvantage for clinical application. Additionally, MTA is expensive.[10] A systematic review of dental pulp capping agents found that pure calcium hydroxide powder has the same biocompatibility as MTA. However, another study reported failures when calcium hydroxide cement was used.[11] Additionally, calcium hydroxide powder and its modifications, including varying particle size and combination with a natural source to enhance its antioxidant activity, have been shown to reduce pulp tissue inflammation and increase the calcium properties of materials.[12–14]

The efficacy of calcium hydroxide as a reparative dentin inducer can be enhanced through the implementation of various optimization techniques. These include a nanoscale escalation of anti-inflammation, calcium release and particle reduction. Calcium hydroxide dissolves into calcium ions and hydroxyl ions. The hydroxyl ions produce an alkaline environment, neutralizing the acidic pH caused by inflammation. Furthermore, they aid in the resolution of inflammation and the progression of dentin reparative formation. However, due to the high alkalinity of the environment, calcium hydroxide also causes coagulation necrosis.[6,12,13] Cocoa pod husks are a natural product that has been studied for their flavonoid and methylxanthine content, as well as their ability to induce reparative dentin when combined with calcium hydroxide. A previous study found that the combination of cocoa pod husks with calcium hydroxide resulted in the formation of a greater quantity of reparative dentin than when calcium hydroxide was used alone.[14]

Calcium ions (Ca^{2+}) play a regulatory role in the initiation of cellular events that lead to tertiary dentinogenesis. After dental pulp cells differentiate into odontoblast-like cells, they secrete extracellular matrix (ECM) and protein. A large influx of Ca^{2+} and phosphorus (P) ions is necessary for the mineralization of the collagenous ECM and the formation of hydroxyapatite.[15] Mineral trioxide aggregate has advantages over calcium hydroxide in terms of the quantity and consistency of calcium released, which provides biocompatibility and sealing ability.[6] The addition of anchovy powder can optimize the calcium release of calcium hydroxide. Anchovies are a rich source of calcium and are distinct from other calcium sources in that they do not contain substances that interfere with absorption or cause allergic reactions.[16]

The use of nanoscale combination materials has been shown to increase surface area, reaction rate, dissolution rate, and bioavailability.[17] Reports have indicated that the application of nanotechnology facilitates the distribution of active molecules, lowers the inflammation rates and enhances dental pulp cell differentiation.[18]

In our study, we examined the characteristics of a nano-combination of calcium hydroxide, cocoa pod husk extract and pure anchovy powder. The optimal formulation was identified based on the viscosity, setting time, pH, solubility, antioxidant activity, and cytotoxicity.

Material and methods

Preparation of a nano-combination of calcium hydroxide, cocoa pod husk extract and anchovy powder

Nano-calcium hydroxide (80–100 nm) (Nanoshel, Punjab, India) was used in the experiment. The cocoa pod husks were obtained from a local producer (Kampung Coklat, Blitar, Indonesia). The fresh anchovies were procured from the fish market in Muara Karang, Jakarta, Indonesia. The cocoa pod husk extract and the anchovy powder used in the research were in nanoscale particles. Each of the natural materials was prepared to optimize the ability of calcium hydroxide to induce reparative dentin.[19] The nanopowder of the cocoa pod husk extract was prepared using ultrasonic-assisted extraction with 80% ethanol, followed by freeze-drying for 48 h to produce a particle size of 107.8 ±27 nm. Similarly, the anchovy nanopowder was prepared by high-energy milling for 24 h, resulting in

a particle size of 789.3 ±170.7 nm. The mixture of nano-calcium hydroxide, cocoa pod husk extract and anchovy powder was prepared in weight ratios of 1:1:1 (group 1), 2:1:1 (group 2), 1:2:1 (group 3), and 1:1:2 (group 4), as well as in the pure nano-calcium hydroxide solution (control group). The materials were weighed on an analytical balance (FS-AR210; Fujitsu, Tokyo, Japan). The powder mixtures were homogenized using mixer mills (MM 400; Retsch, Munich, Germany). For each of the subsequent tests, the combination powder from each group was mixed with sterile aquadest (Sigma-Aldrich, St. Louis, USA) into a paste. The ratio of powder to liquid was 1:1.[17] To ensure thorough homogenization, the powder and liquid were mixed for 30 s in a mixing capsule using an amalgamator (Ultramat 2; SDI Limited, Victoria, Australia).[20]

Viscosity test

The viscosity test was performed with the use of a viscometer (Brookfield HAT; Anderen Ltd, Stoke-on-Trent, UK). The glass beaker was filled with approx. 200 mL of the paste and was placed beneath the tool. The tool was lowered until the spindle was fully submerged. The test was carried out at various rotation speeds. The reading on the dial was multiplied by the correction factor in order to calculate the viscosity.[21] The test was repeated 6 times for each group. The viscosity was necessary for the low value.

Setting time examination

The initial and final setting times were examined using Gillmore needles (Dental Material and Testing Center of Research and Education, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia). The tool consisted of 2 arms with needles that differed in weight and tip diameter. A needle with a weight of 100.0 ±0.5 g and a tip diameter of 2.0 ±0.1 mm was used to determine the initial setting time. A 456.0 ±0.5 g and 1.0 ±0.1 mm-tip-diameter needle was used to evaluate the final setting time. The sample paste was initially placed in a ring mold (10.0 ±0.1 mm internal diameter, 2.0 ±0.1 mm thickness), and the mold was then positioned beneath the initial needle. The needle was lowered until it was fully submerged in the paste, held for 5 s, and then raised. The needle was lowered every 30 s, and the initial setting time was obtained when the needle was unable to make a full circular indentation in the inspected sample. The investigation was continued in the same manner with the second needle to obtain the final setting time.[22] The test was repeated 6 times for each group. The optimal setting time was identified as the shortest setting time.

pH determination

The sample was placed in a cylindrical mold with a diameter of 10.0 ±0.1 mm and a height of 2.0 ±0.1 mm. Six specimens were created for each group. Each specimen was placed in a vial containing 10 mL of distilled water and incubated at 37°C.[23] The pH of each group was determined at 3 h and 24 h using a pH meter (Oakton pH 2700 Benchtop Meter; Cole-Parmer, Vernon Hills, USA). The electrode was calibrated before being immersed into the sample paste. The displayed value was recorded. The test was conducted in triplicate for each sample, and the mean data was recorded as the pH value. The optimal pH was determined for materials with high alkalinity.

Solubility test

The samples were prepared and placed in 6 molds with dimensions of 20.0 ±0.1 mm in diameter and 1.5 ±0.1 mm in height. The mold was placed on a glass slab, filled with the paste, and flattened with a second glass slab. Six specimens were created for each group. The specimens were incubated at 37°C for 24 h and then exposed to air for 15 min. Then, they were weighed in triplicate using an analytical scale (FS-AR210; Fujitsu), with the mean data used as the sample weight (S). Bottle glasses were prepared and weighed 3 times, with the mean data utilized to calculate the bottle weight (W_0). The specimen was placed in a bottle glass containing 5 mL of distilled water and incubated at 37°C for 24 h. This was followed by a drying process, which commenced with rinsing the bottle containing the specimens with distilled water and then drying in an oven at 105°C. The bottle and residues were weighed on the analytical scale (FS-AR210; Fujitsu) 3 times for each specimen. The mean data was used to calculate the W_0 and the residue weight (W_t). The solubility was calculated by dividing the difference between W_t and W_0 by S and multiplying the result by 100. The optimal solubility was obtained if the value was less than 3%.[23]

Antioxidant assay

The antioxidant assay was conducted at the Research Center for Chemistry (Indonesian Institute of Sciences, Jakarta, Indonesia) using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. A total of 4 mg of each sample was weighed and diluted with methanol to create a 1000-L solution. The tests were carried out with various concentrations of 10%, 50%, 100%, and 200%. To the solution, 0.5 mL of DPPH was added, mixed and allowed to stand for 30 min. The blank solution was prepared by adding 0.5 mL of methanol to 2 mL (0.2 mM) of DPPH solution. An ultraviolet-visible (UV-Vis) spectrophotometer (UH5300; Hitachi, Tokyo, Japan) was used to measure the absorbance at 517 nm. The difference between the blank and the sample was used to calculate sample inhibition. The analysis was conducted in duplicate. The antioxidant activity was classified as inactive (IC_{50} > 500 µg/mL), weak (>250–500 µg/mL), moderate (>101–250 µg/mL), active (50–100 µg/mL), or highly active (<50 µg/mL).[24]

Cytotoxicity assay

The cytotoxicity of the optimal ratio to human dental pulp stem cells (hDPSCs) was examined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at PT Prodia StemCell (Jakarta, Indonesia). First, the mixture powder (calcium hydroxide, cocoa pod husk extract and anchovy powder) was combined with distilled water in a 1:1 ratio to achieve the optimal ratio combination. The paste was then placed into a cylindrical mold (25 mm in diameter and 5 mm in height) and kept at room temperature for 24 h. Following this period, the sample was removed from the mold, placed into a 1-mL culture medium, and incubated at 37°C for 24 h. Subsequently, the medium of the samples was filtered (0.1 µL) and stored at –20°C. An indirect cytotoxicity assay was performed according to the ISO 10993 standard.[25] The hDPSCs were placed onto 96-well plates at a density of 1×10^4 cells per well and cultured in a growth medium for 24 h. A series of dilutions of the sample medium was prepared at concentrations of 100%, 50%, 25%, and 10%. After 24 h, an MTT assay was performed, and the percentage of cellular growth was calculated. The cells in the growth medium served as the control group.[25]

Statistical analysis

The data was presented as mean (*M*) and standard deviation (*SD*). A one-way analysis of variance (ANOVA) was employed for each test using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). The level of significance was set at $p < 0.05$. After obtaining the results of the statistical analysis, the group with the optimal ratio was selected for the assessment of the antioxidant and cytotoxic properties.

Results

Viscosity

As shown in Fig. 1, the control group (nano-calcium hydroxide) exhibited the lowest viscosity, followed by group 1. One-way ANOVA revealed statistically significant differences between the groups ($p < 0.01$).

Initial and final setting times

The initial and final setting times of the material are shown in Fig. 2. Group 1 and group 4 showed a shorter initial setting time than the control group. Groups 2 and 3 demonstrated longer initial and final setting times than those observed in the control group. The mean value of the initial and final setting times for group 1 was consistently lower than that of the control group. The one-way ANOVA indicated a significant difference between

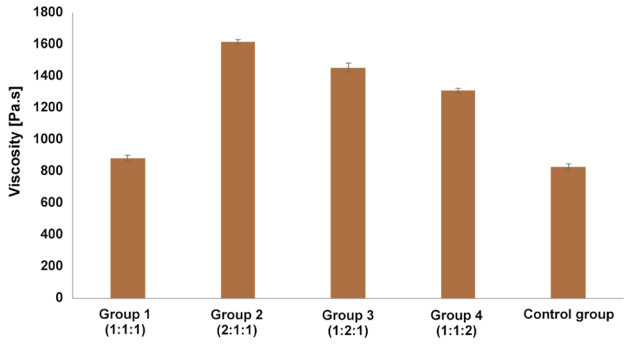

Fig. 1. Viscosity of the nano-combination of calcium hydroxide, cocoa pod husk extract and anchovy powder at varying ratios and the control group

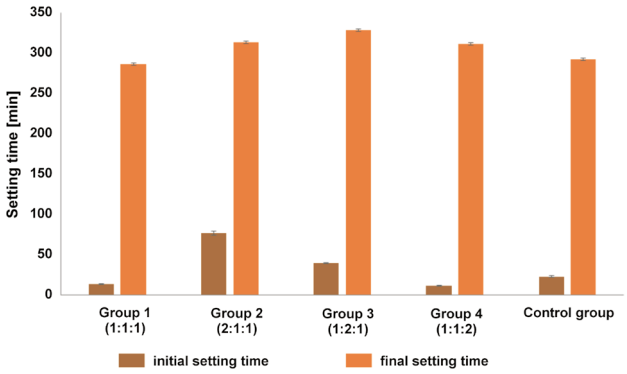

Fig. 2. Initial and final setting times of the nano-combination of calcium hydroxide, cocoa pod husk extract and anchovy powder at varying ratios and the control group

the initial and final setting times in all groups ($p < 0.01$). Furthermore, Tukey's test showed significant differences between the groups in the initial and final setting time results ($p < 0.01$). However, no statistically significant difference was observed between groups 2 and 4 in the final setting time ($p = 0.107$).

pH values

Figure 3 illustrates the comparison of pH values of the samples after 3 h and 24 h. The control group showed the highest pH values, followed by group 1. One-way ANOVA indicated a statistically significant difference in pH between 3 h and 24 h in all groups ($p < 0.001$). Furthermore, Tukey's test did not reveal any significant differences between groups 2 and 4 after 3 h ($p = 0.311$). Additionally, no significant differences were observed between the 24-h pH values in groups 1, 2 and 3 ($p > 0.05$). All the tested materials exhibited pH values above 8.5.

Solubility

As shown in Fig. 4, the solubility of the control group was 4.41%. Group 3 had a lower solubility than the control group. In contrast, the remaining groups demonstrated a higher solubility than the control group.

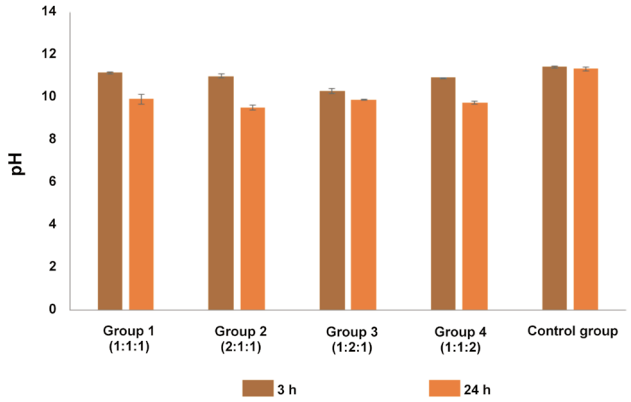

Fig. 3. pH values of the nano-combination of calcium hydroxide, cocoa pod husk extract and anchovy powder at 3 h and 24 h, with varying ratios, and the control group

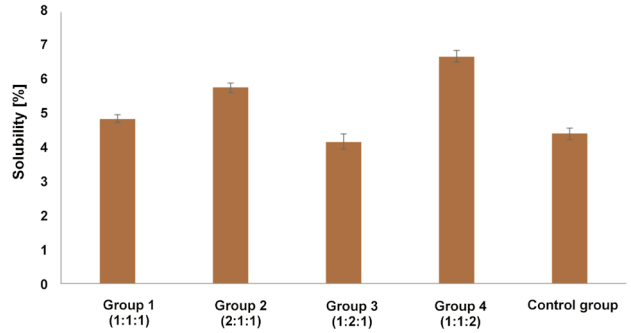

Fig. 4. Solubility of the nano-combination of calcium hydroxide, cocoa pod husk extract and anchovy powder at varying ratios and the control group

One-way ANOVA followed by Tukey's test revealed that there were no statistically significant differences between group 3 and the control group ($p = 0.113$). The solubility rate of all tested materials exceeded 3%.

Selection of the group exhibiting the optimal properties

According to the evaluation of the viscosity, setting time, pH, and solubility, group 1 possessed the most favorable properties. The 1:1:1 ratio displayed the lowest viscosity, the shortest final setting time, a 24-h pH range of 8.5–10.5, and was second in the solubility rate compared to the other groups. Accordingly, the ratio was considered optimal and was subsequently evaluated for its antioxidant and cytotoxic properties.

Antioxidant property of the optimal ratio

The DPPH assay at the half maximal inhibitory concentration (IC_{50}) of the optimal ratio combination of 3 nano-scale materials was determined to be 62.21 ±0.10 µg/mL. Given its strong antioxidant activity, quercetin was used as a comparison standard, displaying a scavenging activity of 4.50 ±0.00 µg/mL.

Cytotoxicity of the optimal ratio

Figure 5 and Table 1 present the results of the viability test. All concentrations of the optimal ratio combination showed no evidence of cytotoxicity. Conversely, the more concentrated the sample, the more proliferative the activity of the hDPSCs that were induced. There was no statistically significant difference between the 10% concentration and the control group ($p > 0.05$).

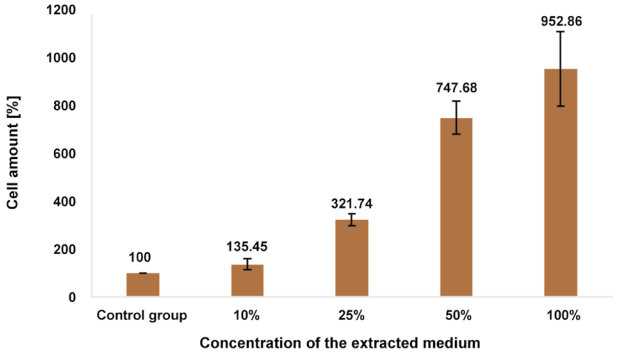

Fig. 5. Cell viability of the optimal ratio of calcium hydroxide, cocoa pod husk extract and anchovy powder nano-combination, and the control group

Table 1. Impact of the optimal ratio of calcium hydroxide, cocoa pod husk extract and anchovy powder nano-combination on cell viability

Concentration	Cell viability *M* ±*SD*	*p*-value (compared to the control group)
10%	135.45 ±22.35	0.281
25%	321.74 ±25.03	0.034*
50%	747.68 ±68.32	<0.001*
100%	952.86 ±155.38	<0.001*

* statistically significant ($p < 0.05$, analysis of variance (ANOVA)); *M* – mean; *SD* – standard deviation.

Discussion

In the presented research, we conducted an analysis to identify the optimal ratio of the nano-combination of calcium hydroxide, cocoa pod husk extract and anchovy powder. Each of the natural elements had the potential to enhance the calcium hydroxide capacity by facilitating the formation of the reparative dentin of a good quality. The flavonoids and theobromine found in cocoa pod husks have antioxidant properties, which result in an anti-inflammatory effect. Additionally, they function as an antibacterial agent.[25–31] In a study that compared the application of calcium hydroxide and cocoa pod husk combination to pulp perforation in rats, the calcium hydroxide effect was improved.[14]

The study demonstrated that the combination resulted in the formation of a greater quantity of reparative dentin than when pure calcium hydroxide was used alone.[14] Another approach used in the present study was the addition of anchovy powder. The application of anchovy has been shown to induce odontoblast proliferation in rat dental pulp, whereas reports have indicated that anchovy cream application resulted in the formation of more reparative dentin than when pure calcium hydroxide was used alone.[32,33] Nanoscale materials were used to optimize the desired effect. It is established that nanotechnology can optimize the distribution of bioactive molecules necessary for reducing inflammation and inducing the differentiation of pulp cells.[18,34] Nanoscale materials range in size from 1 nm to 1000 nm, although many scientists restrict this range to <100 nm.[35]

Previous research showed that direct pulp capping materials require a setting time, solubility and pH properties.[22] Another study concentrated on the pH and solubility characteristics.[23] Therefore, we focused on the above properties when analyzing the nano-combination of calcium hydroxide, cocoa pod husk extract and anchovy powder.

The viscosity of the testing materials should be low, given that they are intended to serve as reparative dentin inducers. Additionally, the materials must exhibit good flow properties to adapt to irregular cavity floors. The low viscosity of the material will prevent the formation of gaps and postoperative sensitivity. It should be noted that there is currently no standard viscosity for such materials.[36] The mean data from group 1 (1:1:1 ratio) revealed the lowest viscosity (884.17 ±20.59 Pa.s) among the tested groups, with the exception of the control group. The obtained viscosity result was considered high due to the nanoparticle size of the materials. The standard powder and liquid ratios were analyzed in this study. Similarly, nanosized particles yielded a greater number of particles than microsized particles and increased the viscosity of the testing materials.[37]

The setting time was divided into 2 parts: initial and final. The initial setting time started with the operator beginning to mix powder and liquid components and concluded when the first Gillmore needle was unable to make a full indentation. The final setting time was calculated by adding the initial setting time to the time required for the second Gillmore needle to make a full indentation.[22] The results revealed that the materials exhibited a prolonged setting time. As previously reported, there is currently no standard for this parameter.[36] However, knowledge of the setting time allows for the anticipation of clinical procedures and the prediction of the working time. In our study, group 4 (ratio 1:1:2) showed the shortest initial setting time (11.42 ±0.49 min), and group 1 (ratio 1:1:1) showed the shortest final setting time (286.33 ±1.40 min). The initial setting time was shown to be shorter than MTA (70 min), as reported by previous study.[20] According to the same study, the initial and final setting times were not fixed when the restoration procedure was performed, as they were influenced by material rheology. In the case of MTA, restoration can be completed in 9.5 min before the initial setting time.[20] The high viscosity of the MTA may reduce the time required for the restoration procedure.

The pH value of the testing materials affects the signaling of molecules in inflamed tissues. It is acknowledged that high-pH calcium hydroxide causes coagulation necrosis on the tissue surface upon contact with the material.[6,13] The results of the present study demonstrate a pH value for calcium hydroxide of 11.43 ±0.07, which was consistent with the range reported in other studies (11.5 ±0.2).[38] In our study, no significant difference was observed between the 3-h and 24-h time points, indicating that the pH value remained relatively stable during this period. All tested materials exhibited a pH value greater than 8.5. The high pH level activated the transient receptor potential ankyrin 1 (TRPA1), which promoted dentinogenesis in the odontoblast. Even in the absence of Ca^{2+}, the high alkalinity (pH 9) improved store-operated Ca^{2+} entry (SOCE).[39] In another report, dental pulp cells cultured at pH 7.5 caused growth arrest or cell death, whereas those cultured at pH 9 showed moderate proliferation.[40] This implies that all tested materials had a favorable pH value.

In a study by Poggio et al., the material solubility was expected to be less than 3%.[23] However, the authors showed that the solubility was above 3%. Although all tested materials did not meet the specified parameters, the standard was more applicable to ready-made materials. The solubility of pure calcium hydroxide or its modifications promoted the dissolution of bioactive material into the tissue.[23] In our study, the lowest solubility was observed in group 3 (1:2:1) (mean: 4.17 ±0.22%), followed by group 1 (1:1:1) (mean: 4.85 ±0.11%). Group 1 demonstrated the most optimal ratio of nano-calcium hydroxide, cocoa pod husk extract and anchovy powder based on the 4 parameters.

The antioxidant property of the material was evaluated based on the subsequent anti-inflammatory reaction. Prior research has shown that the inflammatory process augments the release of reactive oxygen species (ROS) and inflammatory mediators, which in turn increases signal transduction and mediates critical cellular stress responses. Reactive oxygen species are produced throughout the process, resulting in cell destruction and subsequent impact on cell viability. The antioxidant aids in disrupting the cycle and creates a balanced environment for repair.[41] In our study, the IC_{50} of a nano-combination of calcium hydroxide, cocoa pod husk extract and anchovy powder was 62.21 ±0.10 μg/mL. The antioxidant activity of the cocoa pod husk was determined, and the result was consistent with those previously reported for the cocoa pod husk (58.014 ±0.004 μg/mL).[26] The antioxidant activity was confirmed.[24]

Unlike pure calcium hydroxide, the nano-combination of calcium hydroxide, cocoa pod husk extract and anchovy powder was expected to stimulate cell proliferation. The expectation was based on reports related to anchovy powder[42] and the high pH. The results of the cytotoxicity assay were unexpected. In comparison to the control group, 10% concentration of the nano-combination induces 1.3-fold proliferation, 25% induce 3-fold proliferation, 50% induce 7-fold proliferation, and 100% induce 9-fold proliferation.

The antioxidant and cytotoxic properties of this nano-combination powder require investigation. The findings encourage further research into the potential use of a nano-combination of calcium hydroxide, cocoa pod husk extract and anchovy powder as a reparative dentin inducer.

Conclusions

The novel nano-combination of calcium hydroxide, cocoa pod husk extract and anchovy powder, proposed in this study at a 1:1:1 ratio, was found to possess the requisite properties to be used as a reparative dentin inducer. The formulation promotes the increased rate of restoration placement after treatment, the short initial setting time (13.5 min) and a high pH at 24 h (9.91), thereby promoting tissue healing. The antioxidant properties were identified as active, and the materials demonstrated excellent biocompatibility, as evidenced by the MTT assay. Further basic follow-up tests should be performed in order to prepare the combination, such as an antibacterial potential test. In the future, in vivo research should be conducted to examine the impact of the novel nanostructured material on the dentinogenesis marker. Further evaluation is required to explore the potential enhancement of the material's properties when each component is combined with calcium hydroxide.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Anastasia Elsa Prahasti https://orcid.org/0000-0003-2709-5081
Tamara Yuanita https://orcid.org/0000-0002-5320-7772
Retno Pudji Rahayu https://orcid.org/0000-0003-4429-3653

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Original paper

Mechanical, optical and surface properties of 3D-printed and conventionally processed polyamide 12

Heike Meissner[A,D,F], Marie Vacquier[B,C,E,F], Katarzyna Kresse-Walczak[C,E,F], Klaus Boening[A,E,F]

Department of Prosthodontics, Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Germany

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):729–738

Address for correspondence
Katarzyna Kresse-Walczak
E-mail: katarzyna.kresse@ukdd.de

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on January 26, 2024
Reviewed on March 25, 2024
Accepted on April 2, 2024

Published online on October 31, 2024

Abstract

Background. Polyamide-based materials are suitable for three-dimensional (3D) printing.

Objectives. The aim of the study is to examine the impact of aging on the mechanical, surface and optical properties of polyamide 12.

Material and methods. A total of 116 specimens were examined, comprising 58 conventionally processed polyamide 12 (PA12_C) specimens and 58 3D-printed polyamide 12 (PA12_3D) specimens. The modulus of elasticity was determined before and after mechanical and thermal aging with 1,000, 3,000, 9,000, and 1,000, 3,000 and 7,000 cycles, respectively. The surface roughness (Ra), Ra change (ΔRa) and color change (ΔE) were examined before and after chemical aging (1, 12 and 36 days, with artificial saliva, coffee and red wine) using surface profilometry and color spectroscopy. The Kruskal–Wallis test, Mann–Whitney *U* test and Bonferroni–Holm correction were employed, with a significance level of $p < 0.05$.

Results. Before and after mechanical aging, the modulus of elasticity for PA12_3D showed significantly higher values (761 MPa and 747 MPa, respectively) in comparison to PA12_C (515 MPa and 455 MPa, respectively; adjusted $p < 0.001$). Additionally, before and after thermal aging, the modulus of elasticity for PA12_3D exhibited significantly higher values (833 MPa and 705 MPa, respectively) compared to PA12_C (516 MPa and 458 MPa, respectively; adjusted $p < 0.001$). The Ra of PA12_3D was higher than that of PA12_C at the baseline (0.41 µm compared to 0.31 µm, respectively), and remained higher during the aging process. The ΔRa values were small for both groups. The ΔE was significantly higher for PA12_3D compared to PA12_C after 12 days (6.2 (PA12_3D) compared to 4.8 (PA12_C), adjusted $p = 0.003$) and 36 days of storage in red wine (8.2 (PA12_3D) compared to 6.8 (PA12_C), adjusted $p = 0.003$). After 36 days of coffee storage, the observed changes were found to be statistically significant (8.6 (PA12_3D) compared to 6.7 (PA12_C), adjusted $p < 0.001$).

Conclusions. The 3D-printed polyamide 12 demonstrated higher rigidity, Ra and discoloration compared to the conventionally processed polyamide 12. However, not all of the observed parameter differences were significant or clinically relevant. These differences may impact clasp retention, biofilm formation and aesthetic appearance. Nevertheless, the clinical efficacy of 3D printing may be significant.

Keywords: 3D printing, nylon, polyamide 12, dental clasps, removable denture

Cite as
Meissner H, Vacquier M, Kresse-Walczak K, Boening K. Mechanical, optical and surface properties of 3D-printed and conventionally processed polyamide 12. *Dent Med Probl.* 2024;61(5):729–738. doi:10.17219/dmp/186712

DOI
10.17219/dmp/186712

Introduction

Nowadays, missing teeth can be replaced with removable partial dentures (RPDs).[1,2] They can be used either temporarily, as interim dentures, or permanently, depending on the design and material.[1] Removable partial dentures can be constructed using metal-based or polymer-based frameworks.[1] Polymethyl methacrylate (PMMA) is a widely used polymer for RPDs, although it has certain limitations. These include residual monomer (methyl methacrylate) leakage, making it unsuitable for patients who are allergic to these components.[3] The use of metal-based frameworks with metal clasps is recommended for the fabrication of definitive RPDs.[1] Despite many advantages, applying this technique may compromise aesthetics.[1] To address these concerns, non-metal clasp dentures (NMCDs) were introduced, utilizing clasps made from thermoplastic resins.[1,4] Non-metal clasp dentures are available in 2 variations: rigid, with a metal framework including occlusal rests; and flexible, without a metal framework.[4] Flexible NMCDs have limited indications, one of which is their use as interim dentures (temporary RPDs).[4]

Flexible NMCDs without a metal framework made of polyamide 12 are of interest to researchers due to their aesthetic qualities, biocompatibility and good patient acceptance.[5] In comparison to PMMA and metals, they have a reduced weight and higher flexibility.[3] Thus, they can be easily inserted into small oral cavities (e.g., in pediatric patients, individuals with microstomia, or those with ectodermal dysplasia).[6] Because of the high flexibility and a lower modulus of elasticity, polyamide 12 dentures are resistant to fractures.[3] Therefore, they are considered a potential alternative for selected indications and patients allergic to methyl methacrylate.[3,5–7] However, despite these advantages, they are difficult to reline and polish, especially in a dental office.[6] Flexible NMCDs made of polyamide 12 have also shown potential to traumatize supporting tissue and increase bone resorption.[4,5] However, finite element analysis demonstrated a reduction in stress for the clasps and for abutment teeth when polyamide 12 clasps were used.[8] The absence of longitudinal studies has resulted in a lack of consensus regarding wider indications and extended wearing duration.[4,5] Compared to conventional temporary dentures, such as RPDs made from PMMA, the processing costs of NMCDs are higher. Consequently, cost-effective and efficient technologies are of interest.[5]

Polyamide 12 can be processed using either conventional thermoforming or through three-dimensional (3D) printing. The conventional method is both time-consuming and complex.[9] Three-dimensional printing is a type of additive manufacturing methods. Recently, these methods have gained increased attention and have become popular in the field of dentistry.[10] 3D-printed NMCDs made of polyamide 12 offer an attractive manufacturing technique. Fused filament fabrication (FFF) printing is a relatively simple, cost-effective and time-efficient method.[11,12] However, it is not frequently used in the field of dentistry due to its longer printing times and lower resolution compared to other 3D printing methods.[10] However, in terms of the fabrication of medical components, FFF printing is the most prevalent technique worldwide for thermoplastic polymers, such as polyamides.[12] Currently, it is the only commercially available 3D printing method for polyamide 12.

There is a lack of data regarding the material properties of 3D-printed polyamide 12 for use as flexible, temporary NMCDs. In this study, we will use clinically relevant material parameters to examine whether this innovative method has the potential to replace conventional procedures.

The aim of the present study is to determine the difference in material properties between conventionally processed and 3D-printed polyamide 12 in response to mechanical, thermal or chemical aging. The null hypothesis states that there are no differences between the 2 materials with respect to the modulus of elasticity, surface roughness change (ΔRa) and color change (ΔE).

Material and methods

Figure 1 shows an overview of the study design.

Specimen preparation

A total of 116 polyamide 12 specimens were conventionally processed (n = 58) (PA12_C, control; Johannes Weithas GmbH & Co. KG, Lütjenburg, Germany) or 3D-printed (n = 58) (PA12_3D, r.Pod Dual Extruder; Arfona, New York, USA). Polyamide 12 granules (conventional process) or filaments (3D printing) were used. In both cases, the base material was of the same chemical composition (Valplast® International Corp., New York, USA). All specimens were manufactured with dimensions of 40 mm × 10 mm × 2 mm.

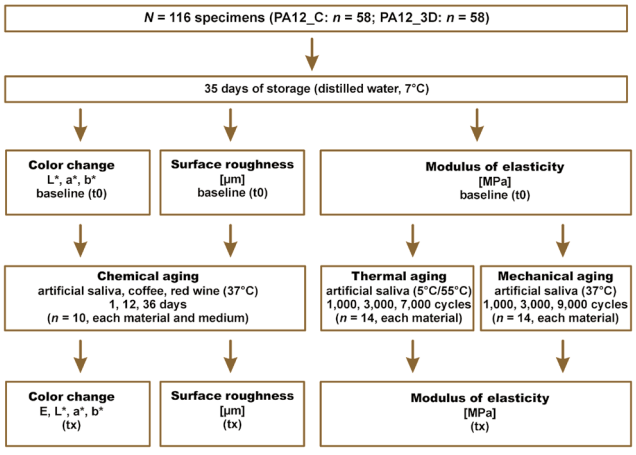

Fig. 1. Study design

PA12_C – conventionally processed polyamide 12; PA12_3D – 3D-printed polyamide; L* – brightness; a* – hue, saturation of the red-green axis; b* – hue, saturation of the yellow-blue axis.

The specimens in the PA12_3D group were produced using FFF printing. The string-shaped polyamide 12 material was subjected to thermoplastic processing in the form of a filament. The material was heated to 220°C through an extrusion nozzle and deposited layer by layer on a build platform in the defined X-Y direction (i.e., along the longitudinal axis of the component). No support material was used during the fabrication of the specimens. The printing path followed a diagonal trajectory at a 45° angle to the X-axis. With a nozzle diameter of 0.4 mm, material layers of 0.3 mm in height were printed with a 100% infill density.

To achieve the optimal surface quality, finishing and polishing were carried out in accordance with the manufacturer's instructions.

The specimens in the PA12_C group (control) were conventionally processed using the thermopress method, utilizing thermoplastic injected polyamide 12 granules. In this process, the granule cartridges in the injection molding machine were heated to approx. 290°C and subsequently injected into the mold at a pressure of approx. 6.5 bar. Post-processing and polishing were carried out following the manufacturer's instructions.

All specimens were stored for 35 days in distilled water at a temperature of 7 ±1°C immediately after production (Med. Kühlgerät MRL 150; Electrolux, Luxembourg City, Luxebourg) to achieve water saturation before testing. The storage temperature was selected to minimize microbial growth during the storage period.

Mechanical aging

The mechanical aging of the PA12_C (n = 14) and PA12_3D specimens (n = 14) was conducted through cyclic compressive loading (Universal Testing Machine Inspekt-Micro LC100N; Hegewald & Peschke, Meß- und Prüftechnik GmbH, Nossen, Germany). The test setup resembled a cyclic three-point bending test in artificial, temperature-controlled saliva at 37°C (UKD saliva solution; University Pharmacy, Dresden, Germany), with a central point load applied to the specimen. The bending cycles were performed at a frequency of 1 Hz, with a displacement of 2 mm, a loading speed of 1 mm/s, and a preload of 1 N. A total of 1,000, 3,000 and 9,000 cycles were performed, which roughly correspond to 1, 3 and 9 years of wear for polyamide NMCDs, respectively.

Thermal aging

The thermal aging was simulated using a thermocycler (THE-1200; SD Mechatronik GmbH, Feldkirchen-Westerham, Germany). The specimens (PA12_C, PA12_3D, n = 14 each) were cyclically exposed to temperature-controlled artificial saliva solution (5°C/55°C, UKD saliva solution; University Pharmacy). Each cycle lasted 77 s, with 27 s in each liquid container and 9 s of drip time. A total of 1,000, 3,000 and 7,000 cycles were performed to simulate 1, 3 and 8 months of use, respectively.

Chemical aging

The PA12_C and PA12_3D specimens were stored for 1, 12 and 36 days at 37°C in artificial saliva (UKD saliva solution; University Pharmacy), coffee (65 g/L water, NESCAFÉ® GOLD; Nestlé Deutschland AG, Frankfurt am Main, Germany) or red wine (pH 3.3, König Arthur Republik Moldau; Andreas Oster Weinkellerei, Cochem, Germany) in a heat chamber (kelvitron® t; Heraeus Instruments, Hanau, Germany). Ten specimens of each material were stored in each medium. A total of 1, 12 and 36 days of storage in the colorants were performed to simulate the intake of beverages over a period of 1 month, 1 year and 3 years, respectively.

Modulus of elasticity

Polyamide 12 was evaluated in accordance with the ISO 20795-1:2013 standard and classified as a denture base polymer, falling under classification type 3: "Thermoplastic molded forms or granules".[13] The modulus of elasticity was determined using a three-point bending test (TIRAtest 2720; TIRA GmbH, Schalkau, Germany). The specimens were subjected to axial loading at a 90° angle across the entire specimen width using a conical indenter (2-mm radius) and a constant test speed of 1 mm/min (preload: 1 N, test endpoint criterion: 10 N). The modulus of elasticity was calculated using the following formula (Equation 1):

$$E = L^2m/4bd^2 \quad (1)$$

where:

E – modulus of elasticity [N/mm^2];

L – support span [mm];

m – gradient of the initial strength-line portion of the load deflection curve [N/mm];

b – width of the specimen [mm];

d – thickness of the specimen [mm].

Surface roughness

The surface roughness was measured with the use of a contact profilometer (Hommel-Etamic W20; JENOPTIK Industrial Metrology Germany GmbH, Villingen-Schwenningen, Germany) and the stylus method. For each specimen, 4 measuring distances were recorded, comprising 2 longitudinal measurements and 2 transverse measurements, each positioned perpendicular to the other. The angle of measurement for the diagonal printing path was 45°. The measurement was

conducted with a stylus tip radius of 2 µm and a tip angle of 90°. The feed mechanism moved horizontally along the surface at a constant speed of 0.5 mm/s and a measuring force of approx. 0.8 mN. The measuring length was 4.8 mm, with a cut-off wavelength of 0.8 mm, and a measurement path of 4.0 m. Subsequently, for each specimen at each measurement time point within the artificial aging process, the arithmetic mean roughness value (Ra) was determined from the 4 measuring distances.

Color change

The color change was determined in accordance with the ISO/TR 28642:2016 standard.[14] For the determination of color stability, the Lab* color system spectrophotometer (Gretag SPM 100; Gretag, Regensdorf, Switzerland) was used. The CIE L*a*b* color space is an approximately uniform color space with coordinates for brightness (L* axis, where L* = 0 represents black and L* = 100 represents white), as well as for hue and saturation (red-green axis (a*), where +a* represents red and −a* represents green; yellow-blue axis (b*), where +b* represents yellow and −b* represents blue). The changes in brightness (ΔL*) and hue (Δa* and Δb*) were defined as differences and were determined for the respective immersion periods and storage media. The individual color parameter differences (ΔL*, Δa* and Δb*) were assessed by subtracting the parameter variables at 2 time points: the measurement time point at baseline (t0) as a specified reference; and after the aging process (tx). The resulting overall ΔE was determined using the following CIE formula (Equation 2):

$$\Delta E = \sqrt{(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2} \quad (2)$$

The color measurements were conducted using a standardized CIE Illuminant D65, which corresponded to natural daylight. The observer angle was set at 2° and was derived from the area of optimal color vision in the human eye. To obtain an average, the measurements were taken at 4 defined points for each specimen. The precise repositioning of the specimens for measuring the L*, a* and b* values at the 4 defined measuring points was ensured through the use of a fixed placement aid on the spectrophotometer. Prior to each measurement, the specimens were thoroughly rinsed with running water and allowed to dry, in order to prevent light reflection.

Statistical analysis

The statistical analysis was conducted using the IBM SPSS Statistics for Windows software, v. 24.0 (IBM Corp., Armonk, USA). The normality of the data was assessed using the Kolmogorov–Smirnov test and the Shapiro–Wilk test. Descriptive statistics were used to summarize the data. For the modulus of elasticity and ΔE, explorative inference statistics were calculated using the Kruskal–Wallis test, given that the data was not normally distributed. A non-parametric Mann–Whitney *U* test was performed to examine differences between the groups. To account for multiple tests, the Bonferroni–Holm correction method was applied to adjust the *p*-values. The alpha level was set at 5%.

Results

Modulus of elasticity after thermal and mechanical aging

Table 1 presents the descriptive data for the modulus of elasticity values following thermocycling and mechanical aging. In both aging methods, the modulus of elasticity for PA12_C showed significantly lower values compared to PA12_3D at all measured time points (raw and adjusted $p < 0.001$). Following thermal (7,000 cycles) and mechanical aging (9,000 cycles), the modulus of elasticity decreased in both PA12_C and PA12_3D compared to the baseline.

Table 1. Descriptive data for the modulus of elasticity before and after thermocycling and mechanical aging

Cycles, *n*	Aging	Material	Modulus of elasticity [MPa]			
			median	*IQR*	min	max
0 (baseline)	TC	PA12_C^{Aa}	516	489–537	446	551
		PA12_3D^{Aa}	833	782–897	754	1,000
1,000		PA12_C^{Bb}	522	484–561	458	571
		PA12_3D^{Bb}	824	782–873	778	909
3,000		PA12_C^{Cc}	525	525–541	467	556
		PA12_3D^{Cc}	792	770–867	737	898
7,000		PA12_C^{Dd}	458	442–478	426	498
		PA12_3D^{Dd}	705	678–747	664	794
0 (baseline)	MA	PA12_C^{Ee}	515	468–539	443	565
		PA12_3D^{Ee}	761	734–791	676	887
1,000		PA12_C^{Ff}	464	454–487	422	566
		PA12_3D^{Ff}	775	754–817	713	877
3,000		PA12_C^{Gg}	493	473–505	426	541
		PA12_3D^{Gg}	779	735–818	701	847
9,000		PA12_C^{Hh}	455	442–470	399	528
		PA12_3D^{Hh}	747	738–794	709	864

IQR – interquartile range; min – minimum; max – maximum; TC – thermocycling; MA – mechanical aging; PA12_C – conventionally processed polyamide 12 (control); PA12_3D – 3D-printed polyamide 12. Lowercase superscript letters indicate significant differences when compared to the respective control (raw *p*-value ≤0.05, Mann–Whitney *U* test). Uppercase superscript letters indicate significant differences in comparison to the respective control (adjusted *p*-value ≤0.05, Mann–Whitney *U* test).

Roughness after chemical aging

The Ra values at the baseline for PA12_3D were observed to be high, reaching up to 0.49 µm (interquartile range (*IQR*): 0.37–0.56 µm). The median Ra value for the control group (PA12_C) was 0.31 µm (*IQR*: 0.26–0.36 µm). Table 2 shows the descriptive data for each tested group. In both groups, none of the Ra values fell below the threshold of 0.2 µm, which is the recommended maximum.[15,16]

Table 3 presents the ΔRa after aging. Overall, only minor changes in Ra were observed.

Color change after chemical aging

Figures 2 and 3 show the specimens before and after 36 days of immersion in coffee. A perceptible ΔE could be observed. Figure 4 depicts the specimens following a 36-day storage period in red wine.

Table 4 presents the descriptive data for color change. After 1 day of storage in coffee or wine, the color change was small and below or equal to 1.9.

Table 2. Descriptive data for the roughness (Ra) before and after chemical aging

Storage time [days]	Medium	Material	Ra [µm]			
			median	*IQR*	min	max
0 (baseline)	artificial saliva	PA12_C	0.29	0.23–0.35	0.20	0.37
		PA12_3D	0.45	0.34–0.82	0.31	1.22
	coffee	PA12_C	0.34	0.28–0.41	0.22	0.50
		PA12_3D	0.35	0.33–0.55	0.28	0.59
	wine	PA12_C	0.31	0.26–0.35	0.22	0.67
		PA12_3D	0.49	0.37–0.56	0.32	0.80
1	artificial saliva	PA12_C	0.32	0.24–0.36	0.22	0.42
		PA12_3D	0.43	0.37–0.77	0.32	1.03
	coffee	PA12_C	0.34	0.29–0.42	0.24	0.47
		PA12_3D	0.37	0.34–0.56	0.32	0.62
	wine	PA12_C	0.30	0.29–0.44	0.26	0.61
		PA12_3D	0.49	0.34–0.61	0.33	0.71
12	artificial saliva	PA12_C	0.33	0.23–0.36	0.20	0.38
		PA12_3D	0.52	0.34–0.76	0.33	1.17
	coffee	PA12_C	0.32	0.27–0.41	0.20	0.45
		PA12_3D	0.36	0.28–0.39	0.20	0.70
	wine	PA12_C	0.28	0.25–0.35	0.23	0.74
		PA12_3D	0.47	0.30–0.71	0.27	0.79
36	artificial saliva	PA12_C	0.28	0.22–0.34	0.20	0.47
		PA12_3D	0.51	0.37–0.51	0.31	1.27
	coffee	PA12_C	0.27	0.23–0.42	0.20	0.49
		PA12_3D	0.39	0.34–0.49	0.27	0.72
	wine	PA12_C	0.28	0.24–0.40	0.21	0.59
		PA12_3D	0.47	0.32–0.65	0.31	0.80

Table 3. Descriptive data for the roughness change (ΔRa) after chemical aging

Storage time [days]	Medium	Material	ΔRa [µm]			
			median	*IQR*	min	max
1	artificial saliva	PA12_C	0.01	−0.02–0.04	−0.07	0.14
		PA12_3D	0.00	−0.04–0.02	−0.19	0.08
	coffee	PA12_C	0.00	−0.05–0.04	−0.09	0.09
		PA12_3D	0.02	0.00–0.04	−0.20	0.11
	wine	PA12_C	0.03	−0.01–0.06	−0.20	0.26
		PA12_3D	0.00	−0.05–0.02	−0.09	0.08
12	artificial saliva	PA12_C	0.00	−0.01–0.02	−0.02	0.05
		PA12_3D	0.01	−0.06–0.06	−0.08	0.12
	coffee	PA12_C	−0.01	−0.05–0.02	−0.09	0.04
		PA12_3D	−0.05	−0.09–0.00	−0.20	0.11
	wine	PA12_C	0.00	−0.03–0.04	−0.11	0.08
		PA12_3D	−0.02	−0.08–0.06	−0.11	0.22
36	artificial saliva	PA12_C	−0.02	−0.03–0.01	−0.05	0.13
		PA12_3D	0.02	−0.02–0.07	−0.27	0.28
	coffee	PA12_C	−0.05	−0.10–0.02	−0.15	0.09
		PA12_3D	0.02	−0.01–0.04	−0.13	0.13
	wine	PA12_C	−0.02	−0.06–0.03	−0.11	0.18
		PA12_3D	−0.01	−0.06–0.03	−0.07	0.16

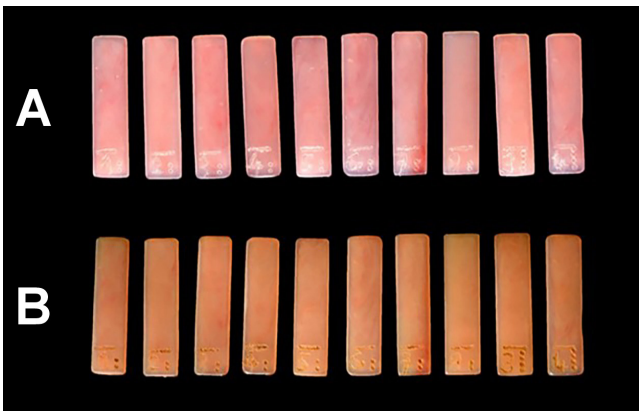

Fig. 2. Conventionally processed polyamide 12 specimens before (A) and after (B) 36 days of storage in coffee

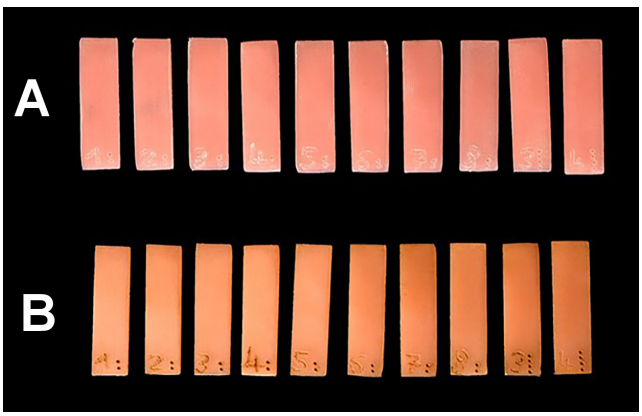

Fig. 3. 3D-printed polyamide 12 specimens before (A) and after (B) 36 days of storage in coffee

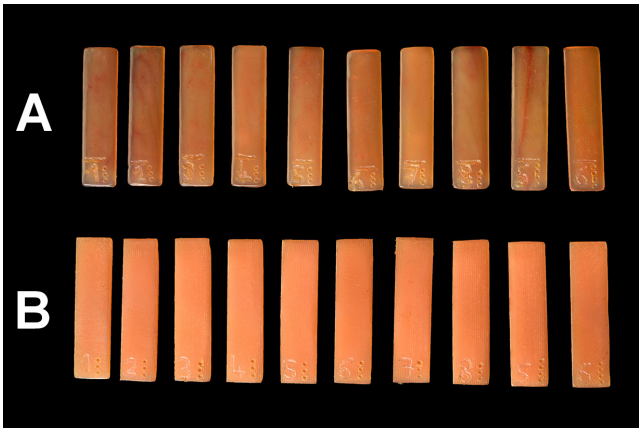

Fig. 4. Conventionally processed (A) and 3D-printed (B) polyamide 12 specimens after 36 days of storage in red wine

Table 4. Descriptive data for color change (ΔE) after chemical aging

Storage time [days]	Medium	Material	ΔE			
			median	*IQR*	min	max
1	artificial saliva	PA12_C	2.7^{Aa}	1.9–3.3	0.8	3.4
		PA12_3D	0.6^{Aa}	0.4–1.1	0.3	5.4
	coffee	PA12_C	1.5	1.3–2.1	1.2	2.6
		PA12_3D	1.6	1.1–2.0	0.7	3.0
	wine	PA12_C	1.7^{Bb}	1.6–1.8	1.4	2.0
		PA12-3D	1.9^{Bb}	1.8–2.1	1.7	4.5
12	artificial saliva	PA12_C	1.3	0.5–2.2	0.2	3.7
		PA12_3D	1.1	0.7–1.5	0.5	1.8
	coffee	PA12_C	3.1	1.3–2.1	1.2	2.6
		PA12_3D	3.5	3.1–4.0	3.0	4.6
	wine	PA12_C	4.8^{Cc}	4.5–4.8	4.1	5.3
		PA12-3D	6.2^{Cc}	5.6–6.3	4.8	6.4
36	artificial saliva	PA12_C	1.7	1.3–2.2	0.4	2.5
		PA12_3D	1.3	0.7–1.5	0.5	1.8
	coffee	PA12_C	6.7^{Dd}	6.3–6.9	6.0	7.2
		PA12_3D	8.6^{Dd}	7.9–9.0	7.7	10.0
	wine	PA12_C	6.8^{Ee}	6.6–7.0	5.9	7.5
		PA12_3D	8.2^{Ee}	7.8–8.9	6.9	9.7

Lowercase superscript letters indicate significant differences when compared to the respective control (raw *p*-value ≤0.05, Mann–Whitney *U* test). Uppercase superscript letters indicate significant differences in comparison to the respective control (adjusted *p*-value ≤0.05, Mann–Whitney *U* test).

After 12 and 36 days of storage in coffee or wine, the highest recorded value for color change was 8.6. A significant increase in ΔE was observed for PA12_3D in comparison to PA12_C after 12 and 36 days of storage in wine (raw $p = 0.001$, adjusted $p = 0.003$). Significant differences were observed for PA12_3D after 36 days in coffee (raw and adjusted $p < 0.001$). In contrast, when immersed in artificial saliva, all median values remained below or were equal to 2.7.

Discussion

The study aimed to detect differences in the material properties of conventionally processed and 3D-printed polyamide 12 following exposure to mechanical, thermal and chemical aging at various time points. The popularity of additive manufacturing has increased in the field of dentistry. Given the differences between conventional and additive manufacturing methods, it is possible that the material properties of the end products differ, even with the same chemical composition. Additionally, the additive manufacturing of polymers is still a developing field.[17] Thus, the evaluation of the properties of these materials is of high importance for the clinician.

The results of this study implied that PA12_3D had a higher modulus of elasticity and, thus, higher rigidity and Ra when compared to PA12_C. Furthermore, PA12_3D showed a higher degree of discoloration after storage in wine and coffee for 12 and 36 days.

During the mechanical and thermal aging tests in the laboratory, it was important to simulate clinical conditions over time. It is essential to investigate the potential for material fatigue induced by repetitive clasp deflection when removing RPDs for cleaning, as this could lead to loss of retention and damage of clasps. The repair of polyamide 12 dentures is a challenging process.[7] In our study, we simulated a scenario of 1, 3 and 9 years of wearing polyamide NMCDs for the removal and insertion of the denture. These periods were calculated on the assumption that patients would remove their dentures twice a day. It can be assumed that 3,000 and 9,000 cycles represent the upper limits of durability for temporary dentures. However, in real life, prolonged use should be taken into consideration. To simulate the physiological intraoral conditions during thermal aging, an approximate temperature change of 20 to 50 thermal cycles per day was assumed. Hence, 10,000 thermal cycles corresponded to a wearing time of 1 year.[18,19] In our experiments, thermal cycling corresponds to an approximate wearing time of 1, 3 and 8 months, which is typical for temporary dentures.

The staining of dentures may cause significant aesthetic drawbacks and is attributed to the consumption of food and beverages.[19,20] Consequently, in vitro staining simulations are crucial in anticipating potential color changes during daily use. The storage of dentures in wine and coffee for periods of 1, 12 and 36 days may roughly simulate the consumption of these products over 1 month, 1 year and 3 years, respectively.[21]

Coffee and wine are often used to simulate discoloration.[21–23] Coffee contains tannic acid and yellow colorants that are responsible for color changes.[22] It also has a higher discoloration potential than tea.[22] For red wine, the low pH and ethyl alcohol content may be responsible for discoloration.[22] As previously mentioned, denture use may be prolonged, therefore, simulating 3 years of use aims at a worst-case scenario.

The modulus of elasticity was tested during mechanical and thermal aging as an indicator of clasp longevity. The null hypothesis was rejected, as the results demonstrated significant differences between both processing methods at all tested time points. The significant difference between the 2 methods remained consistent throughout the course of both aging processes. With regard to the maximum number of cycles tested for both aging methods, the modulus of elasticity decreased slightly in comparison to the baseline.

In this study, the modulus of elasticity for PA12_C was found to be approx. 300 MPa lower than that of PA12_3D. Additionally, it was 500 MPa lower than the values reported in prior studies for conventionally processed material.[22,24,25] For example, Wieckiewicz et al. reported initial values of approx. 1,000 MPa for dry specimens.[22] This discrepancy can be attributed to the water storage and sorption of conventionally processed polyamide 12 (Valplast® International Corp.). This results in a reduction in the flexural strength and modulus of elasticity.[25] It is evident that water molecules permeate the polymer structure, acting as plasticizers that enhance the mobility of polymer chains.[25] The impact of water storage, ranging from 2 days (50 ±2 h) to 30 days at 37°C, resulted in a one-third reduction in the modulus of elasticity.[24,25] The changes in the modulus of elasticity after water sorption have clinical relevance to the retention force.[25] To simulate the clinical use and obtain more accurate results, it was crucial to store the specimens in water before conducting the tests. Moreover, additional differences in experimental design among the referenced studies result in varying experimental conditions. This discrepancy may affect the comparability of the obtained results.

The modulus of elasticity is a critical parameter in the evaluation of dental materials used in clasp manufacturing. Materials with a high modulus of elasticity (>2,000 MPa), such as cobalt–chromium (CoCr) alloys, are recommended for clasp and RPD framework designs.[26] Polyamide 12, on the other hand, has a substantially lower modulus of elasticity. Thus, clasps manufactured from polyamide 12 are more flexible and may use larger undercuts on abutment teeth for retention, which would have been leveled for metal clasps.[24] Flexible clasps facilitate less stressful denture insertion for abutment teeth and reduce stress in the retentive arm of the clasp compared to metal clasps.[8] However, the flexibility of the denture base is a concern, as it can potentially lead to uneven stress distribution on the mucosa and alveolar bone, particularly in the mandible.[4,27]

Our study showed that PA12_C had a significantly lower modulus of elasticity compared to PA12_3D. This discrepancy may be attributed to the different manufacturing methods employed and may be a potential advantage for printed polyamide 12.

Differences could occur using FFF printing. The polyamide 12 filament was melted at a lower temperature compared to conventional processing to ensure dimensional stability. This could result in the emergence of different material properties. The higher rigidity of PA12_3D could be more suitable for use as temporary dentures compared to PA12_C. If the differences in the modulus of elasticity, for example 300 MPa, as observed in our study between conventionally processed and 3D-printed polyamide 12 prove to be clinically significant, further investigation would be warranted.

The higher modulus of elasticity for PA12_3D could have a significant clinical impact, as claps that are too flexible may fail to offer sufficient retention for the RPDs.[28] Furthermore, polyamide 12 clasps require less removal force and exhibit clinically unacceptable retention in small undercuts, when simulated, even with a modulus of elasticity of 1,440 MPa.[8] The lower modulus of elasticity in conventionally processed polyamide 12 clasps can result in inadequate clinical retention. Additionally, contrary to metal clasps, adjusting the retention force after the production of polyamide 12 clasps is not feasible.[4] Nevertheless, Kümbüloğlu et al. observed no significant deformation of conventionally processed polyamide 12 clasps after 36 months of simulated clinical use.[28] Furthermore, a clinical pilot study by Boeckler et al. demonstrated favorable outcomes for clinical use within 6 months.[29] A recent report by Spintzyk et al. showed a clinical application of 3D-printed polyamide non-metal clasp RPDs with acceptable fit and sufficient retention.[30]

In the qualitative assessment of denture base materials, the measurement of the Ra is of particular interest. The Ra value is largely dependent on the preceding polymerization and processing procedures, as well as the subsequent finishing and polishing.[31] To ensure comparable conditions for the Ra measurements, standardized polishing protocols were conducted in accordance with the manufacturers' instructions. The Ra for PA12_3D was consistently higher than that of PA12_C at all measurement time points and for all tested media. This discrepancy is likely attributable to the different manufacturing processes. Fused filament fabrication has a low resolution. The molten filaments are printed layer by layer. Even after the layers have been merged, they can still be identified. Furthermore, grinding and polishing proved ineffective in eliminating the inner layer-wise structure.[30]

Nevertheless, a slight change in Ra values was observed during the chemical aging process, and its clinical relevance remained uncertain. This finding was consistent with other literature results that demonstrated no influence on ΔRa using thermal or chemical aging, even when treated with aggressive chemical agents such as glutaraldehyde or sodium hypochlorite.[4,32]

Regarding Ra, all specimens showed Ra values that were above or equal to the recommended maximal threshold of 0.2 μm.[15,16] The ΔRa between the 2 processing methods was minimal and may be considered clinically irrelevant. Therefore, no interferential statistics were used to compare both processing methods.

The presence of rough surfaces on clasps and denture base materials promotes the accumulation of plaque and bacterial adhesion.[6] As a clinical consequence, the risk of denture stomatitis or caries at abutment teeth may be elevated.[6] High roughness on retentive clasp surfaces is considered unfavorable, with a smooth surface being the desired target.[33,34] Proteoglycans in human saliva are effective lubricants. Thus, it can be assumed that rough surfaces of denture clasps do not contribute to clasp retention. It is therefore recommended that improvements be made in the production or polishing process.

Regarding color changes, the null hypothesis was found to be partially rejected, showing significant differences between both processing techniques at various time points and in the presence of different chemical aging media.

The specimens derived from both manufacturing processes exhibited a similar tendency towards discoloration. Surprisingly, PA12_C showed the highest ΔE after 1 day when stored in artificial saliva. This discrepancy in color change did not fit the overall data and may be attributed to measurement error. The most significant discoloration was observed following exposure to red wine and coffee, particularly when simulating more than 1 month of use. A color change of 3.7 is regarded as the threshold recommended for gingival shades.[35,36] The color changes observed when simulating 1 year of storage in wine and 3 years in coffee or wine, respectively, exceeded this threshold. Perceptible color changes could pose substantial aesthetic concerns for patients and affect their satisfaction with the treatment.[20,35–38] There is an established association between the color of artificial teeth and the utilization of RPDs.[39] Additionally, it is important to note that an increased dissatisfaction with tooth appearance is related to a dissatisfaction with tooth color and is significantly linked to the desire for treatment.[40] Patients expressing dissatisfaction with tooth color may be susceptible to discoloration of the polyamide clasp color. Furthermore, patients under the age of 65 are more likely to experience aesthetic dissatisfaction.[39] Given that RPDs, especially temporary dentures, are utilized across all patient groups, good color stability is crucial in dental materials, particularly in polyamide clasps.

The results of this study are in line with those previously reported by Wieckiewicz et al. regarding discoloration, indicating a need for material improvement.[22] A potential avenue for improvement entails modifications to the printing process. Another potential solution is the application of a sealant agent. A recent study showed that this treatment could markedly reduce the color change of polyamide 12.[41] Reducing the color change could have a positive impact on the clinical performance of polyamide 12 dentures.

Limitations

One of the limitations of this study was that the same specimens were tested at different time points. If a specimen was damaged at the beginning or during the course of the experiment, the error was carried over all measurement time points. To minimize the potential for damage during the three-point bending test, the end-of-test criterion was carefully selected to ensure that no irreversible material change could have occurred. No tests exceeded the linear section of the curve for the determination of the modulus of elasticity using Hooke's straight line. As previously stated by Hamanaka et al., the proportionality limit of polyamide 12 (Valplast® International Corp.) is reached at a load of 19 N when tested according to ISO 1567 and ISO 1567:1999/Amd 1:2003.[42–44] In our study, the established end-of-test criterion of 10 N corresponded to a specimen deflection of 1.2 mm in the elastic range of polyamide 12. This was below the proportionality limit, and it is unlikely that it had a negative impact on our findings.

A further limitation is the lack of the modulus of elasticity and roughness measurement before saturation with water. Including these data points might facilitate a more comprehensive understanding and interpretation of the data. Additionally, a comparison with other studies could be more straightforward. Nevertheless, the values obtained for dry polyamide 12 have limited clinical implications, as the saturation level simulates the condition in the oral cavity during the wearing period. Only the data obtained from saturated specimens is clinically relevant. The water saturation affects the modulus of elasticity, influencing the rigidity of clasps and denture bases, as well as the retentive force of the clasp.[25] The retention force plays a crucial role in the clinical success of RPDs. This highlights the importance of in vitro conditions that closely resemble clinical scenarios.

Furthermore, since only 2 different manufacturing processes of chemically identical polyamide 12 material were evaluated, the generalizability of the results to different polyamide 12 products is limited. The literature indicates that there are variations in mechanical, physical and surface properties among different polyamide products.[7,25,42] Moreover, the findings are confined to the processing parameters, particularly in the case of 3D-printed polyamide 12, as it has been demonstrated that these can significantly influence the properties of the material.[45] This limitation also restricts the generalizability of the results.

Conclusions

In conclusion, the findings of the present study indicate that there are notable differences in the properties of 3D-printed polyamide 12 compared to those of conventionally processed polyamide 12. The higher roughness and discoloration of the 3D-printed material may prove disadvantageous in clinical applications. Conversely, the higher modulus of elasticity could be advantageous due to the enhanced rigidity. Further studies are required before 3D-printed material can be recommended for clinical use.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Heike Meissner https://orcid.org/0000-0001-8026-2454
Marie Vacquier https://orcid.org/0009-0008-8235-8896
Katarzyna Kresse-Walczak https://orcid.org/0000-0002-0935-1785
Klaus Boening https://orcid.org/0000-0003-4252-1755

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Review

Microbiological aspects of cancer progression: A systematic review conducted according to the PRISMA 2020 guidelines and the Cochrane Handbook for Systematic Reviews of Interventions

Giuseppe Minervini[1,2,D–F], Sahana Shivakumar[3,A], Vincenzo Ronsivalle[4,B], Rocco Franco[5,C,D], Marco Cicciù[4,D–F], Maria Maddalena Marrapodi[6,C–F]

[1] Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India
[2] Multidisciplinary Department of Medical-Surgical and Odontostomatological Specialties, University of Campania "Luigi Vanvitelli", Naples, Italy
[3] Department of Public Health Dentistry, People's College of Dental Science and Research Center, People's University, Bhopal, India
[4] Department of Biomedical and Surgical and Dental Sciences, University of Catania, Italy
[5] Department of Biomedicine and Prevention, University of Rome "Tor Vergata", Italy
[6] Department of Woman, Child and General and Specialist Surgery, University of Campania "Luigi Vanvitelli", Naples, Italy

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):739–746

Address for correspondence
Giuseppe Minervini
E-mail: giuseppe.minervini@unicampania.it

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on November 10, 2023
Reviewed on December 1, 2023
Accepted on February 5, 2024

Published online on October 31, 2024

Cite as
Minervini G, Shivakumar S, Ronsivalle V, Franco R, Cicciù M, Marrapodi MM. Microbiological aspects of cancer progression: A systematic review conducted according to the PRISMA 2020 guidelines and the Cochrane Handbook for Systematic Reviews of Interventions. *Dent Med Probl.* 2024;61(5):739–746. doi:10.17219/dmp/183712

DOI
10.17219/dmp/183712

Abstract

The complex interplay between the gut microbiota, cancer treatments and patient characteristics has emerged as a significant area of research. This study sought to examine these relationships in the context of colorectal cancer (CRC).

A comprehensive search of relevant studies was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines and the Cochrane Handbook for Systematic Reviews of Interventions. The studies included a variety of treatment modalities and microbiological parameters. A data extraction form, designed specifically for this review, was used to assess a range of variables across all studies.

The analysis revealed a multifaceted interaction between the gut microbiota, genetic factors and treatment outcomes. Elderly patients with CRC frequently received single-agent chemotherapy, with outcomes that were comparable to those of younger patients. The presence of tumorigenic bacteria, including *Escherichia coli* and *Bacteroides fragilis*, was associated with early colon neoplasia. Additionally, an abundance of *Fusobacterium* spp. was observed in colonic adenomas, contributing to a pro-inflammatory environment. Although the FcγRIIIa-158 V/V genotype was associated with higher cetuximab-mediated antibody-dependent cellular cytotoxicity (ADCC), no direct influence of FcγR polymorphisms on treatment response was noted. Furthermore, the combination of programmed cell death protein-1 (PD-1), BRAF and MEK inhibition showed favorable response rates. The gut microbiome, especially the presence of *Fusobacterium* spp., had a notable influence on the therapeutic response in CRC.

These findings underscore the role of the gut microbiota and genetic factors in cancer treatment outcomes, emphasizing the potential of a holistic approach to cancer management. Future research should exploit these findings in order to develop microbiota-modulating strategies and personalized medicine approaches for the purpose of improving the efficacy of cancer treatment.

Keywords: gut microbiota, genetic polymorphisms, cancer treatment outcomes, *Fusobacterium*, tumorigenic bacteria

Introduction

In recent decades, cancer has emerged as a major public health issue, representing a significant global burden with complex, multifactorial etiologies contributing to its onset and progression.[1] With the cancer mortality rate rising, resulting in the loss of millions of lives worldwide,[2] there has been minimal progress in reducing this mortality. Accordingly, a comprehensive understanding of the factors that modulate cancer progression is essential for the development of effective therapeutic strategies. The emerging research has begun to elucidate the intricate role of the human microbiome in health and disease, creating a new paradigm in our comprehension of carcinogenesis.[2]

The relationship between microbial entities and neoplastic cells within the bodily ecosystem can be viewed through the lens of evolutionary dynamics.[3] Specifically, the mutualistic interactions between these 2 cellular populations, which enhance their proliferative capacities and their ability to evade immune surveillance, could potentially confer an evolutionary advantage.[4] This suggests that the physiological environment may often favor the survival and propagation of microbial and neoplastic cells that engage in cooperative behaviors, thereby outcompeting those that do not partake in such synergistic interactions. Such cooperation can be stabilized through evolutionary processes, such as positive assortment or partner selection.[5–8]

Colorectal cancer (CRC) is one of the most prevalent malignancies globally, with significant morbidity and mortality rates.[3] The progression of the disease is multifactorial and influenced by genetic, environmental and lifestyle factors. Among these, the role of the gut microbiota, the complex community of microorganisms that inhabit the human gut, has recently received considerable attention in the field of colorectal carcinogenesis.

The gut microbiota plays an integral role in maintaining homeostasis, including nutrient metabolism, the protection against pathogens and the modulation of the immune system.[4] Dysbiosis, defined as an imbalance or alteration of the gut microbiota, has been associated with various pathological conditions, including inflammatory bowel diseases and metabolic disorders. Recent studies have suggested a potential correlation between gut microbiota dysbiosis and CRC.[5,9–11]

The emerging evidence indicates that gut microbiota dysbiosis may contribute to colorectal carcinogenesis through several mechanisms, including the promotion of chronic inflammation, the production of carcinogenic metabolites and the alteration of host immune responses. However, the exact role of gut microbiota dysbiosis in the progression of CRC remains unclear and is a subject of ongoing research.[12–14]

In addition to the role of the gut microbiota, the treatment modality for CRC can also significantly influence the disease progression. The impact of common treatments such as surgery, chemotherapy and radiation therapy, as well as more recent approaches like immunotherapy, on the course of CRC can vary considerably.[15] The interaction between these treatments, the gut microbiota, and their cumulative effect on CRC progression is a complex interplay that is yet to be fully understood.[16]

Despite the growing body of evidence, our understanding of the microbiological aspects of cancer progression remains fragmented.[5] Previous studies have often focused on specific types of cancer or microbial species,[9–11] which has limited our ability to fully map the overall landscape of microbial influence on cancer progression. Furthermore, the inherent complexity of the microbiome, coupled with the influence of various confounding variables such as diet, antibiotic usage and host genetics, introduces additional layers of complexity to these investigations.

In light of the aforementioned context, we conducted this systematic review with the objective of synthesizing the existing literature on the role of gut microbiota dysbiosis in the progression of CRC and the influence of different treatment modalities. This review aims to collate and analyze the current evidence in order to shed light on the diverse ways in which microorganisms may modulate cancer progression.

Material and methods

PRISMA protocol

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 protocol[17] was employed to guide the review process, the schematics of which are shown in Fig. 1.

PECO framework

The Population, Exposure, Comparison, Outcome (PECO) framework was utilized to define the research question and direct the search strategy:

– Population (P): adult patients (>18 years old) diagnosed with CRC;
– Exposure (E): presence of gut microbiota dysbiosis identified through fecal microbiota analysis (e.g., 16S rRNA gene sequencing, metagenomics);
– Comparison (C): adult CRC patients with normal gut microbiota composition and/or those undergoing different treatment modalities (e.g., surgery, chemotherapy, radiation therapy, immunotherapy);
– Outcome (O): progression of CRC measured by validated clinical staging systems.

Database search protocol

The search strategy for this systematic review was designed to identify all relevant studies exploring the

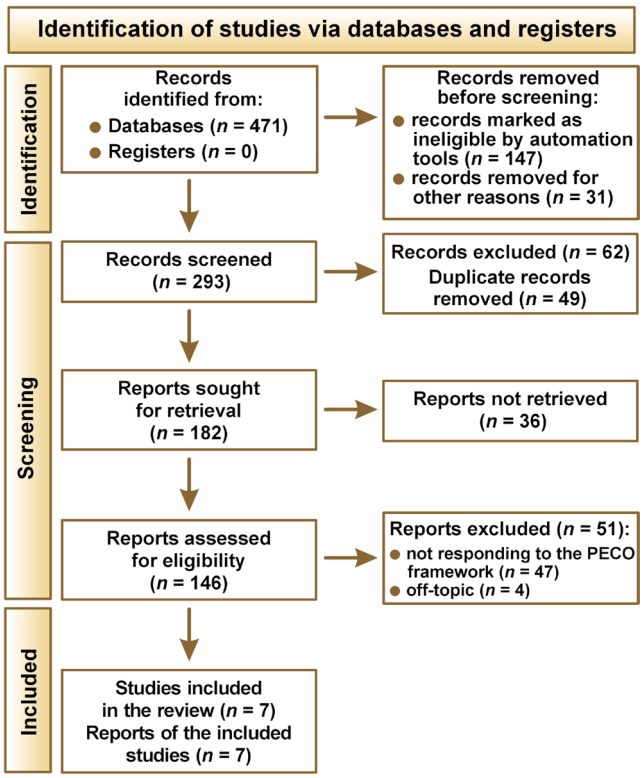

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 flow diagram[17]

PECO – Population, Exposure, Comparison, Outcome.

relationship between gut microbiota dysbiosis and the progression of CRC. A comprehensive search of relevant studies was conducted in accordance with the Cochrane Handbook for Systematic Reviews of Interventions. The search was performed across 8 databases, namely PubMed/MEDLINE, Embase, Web of Science, Scopus, the Cochrane Library, CINAHL, APA PsycINFO, and Google Scholar. The search strategy was then adapted to align with the syntax and subject headings of the other databases, using Medical Subject Headings (MeSH) terms and Boolean operators, as shown in Table 1.

Inclusion and exclusion criteria

The inclusion criteria were as follows: original research studies; studies examining the association between microbiological factors and cancer progression; studies conducted in human subjects; and studies published in English. The following studies were excluded from the review: case reports, case series or animal studies; studies lacking sufficient data on cancer progression; studies not focused on microbiological factors; and reviews, editorials or commentaries.

Data extraction

Two independent reviewers conducted the data extraction using a pre-designed form. Any discrepancies were resolved through discussion or, if necessary, by consulting a third reviewer. The data extraction form captured the following information: the first author's name; the year of publication; the study design; the country where the study was conducted; the sample size; the patient demographics (age and sex); details on CRC diagnosis; the methods used to measure and classify gut microbiota dysbiosis; a description of the comparison group (normal microbiota and/or different treatment modalities);

Table 1. Search strings utilized across the databases

Database	Search string
PubMed/MEDLINE	("Colorectal Neoplasms"[MeSH] OR "colorectal cancer") AND ("Dysbiosis"[MeSH] OR "gut microbiota") AND ("Neoplasm Progression"[MeSH] OR "cancer progression") AND ("Therapeutics"[MeSH] OR "chemotherapy" OR "radiation therapy" OR "surgery" OR "immunotherapy")
Embase	('colorectal tumor'/exp OR 'colorectal cancer') AND ('microbial dysbiosis'/exp OR 'gut microbiota') AND ('tumor progression'/exp OR 'cancer progression') AND ('drug therapy'/exp OR 'chemotherapy' OR 'radiation therapy' OR 'surgery' OR 'immunotherapy')
Web of Science	(TS = ("colorectal neoplasms" OR "colorectal cancer") AND TS = ("dysbiosis" OR "gut microbiota") AND TS = ("neoplasm progression" OR "cancer progression") AND TS = ("therapeutics" OR "chemotherapy" OR "radiation therapy" OR "surgery" OR "immunotherapy"))
Scopus	(TITLE-ABS-KEY ("colorectal neoplasms" OR "colorectal cancer") AND TITLE-ABS-KEY ("dysbiosis" OR "gut microbiota") AND TITLE-ABS-KEY ("neoplasm progression" OR "cancer progression") AND TITLE-ABS-KEY ("therapeutics" OR "chemotherapy" OR "radiation therapy" OR "surgery" OR "immunotherapy"))
The Cochrane Library	("colorectal cancer" in Title Abstract Keyword OR "Colorectal Neoplasms" in MeSH) AND ("gut microbiota" in Title Abstract Keyword OR "Dysbiosis" in MeSH) AND ("neoplasm progression" in Title Abstract Keyword OR "cancer progression") AND ("therapeutics" in Title Abstract Keyword OR "chemotherapy" OR "radiation therapy" OR "surgery" OR "immunotherapy")
CINAHL	(MH "Colorectal Neoplasms" OR TI "colorectal cancer" OR AB "colorectal cancer") AND (MH "Dysbiosis" OR TI "gut microbiota" OR AB "gut microbiota") AND (MH "Neoplasm Progression" OR TI "cancer progression" OR AB "cancer progression") AND (MH "Therapeutics" OR TI "chemotherapy" OR AB "chemotherapy" OR TI "radiation therapy" OR AB "radiation therapy" OR TI "surgery" OR AB "surgery" OR TI "immunotherapy" OR AB "immunotherapy")
APA PsycINFO	(DE "Colorectal Cancer" OR "colorectal cancer") AND ("dysbiosis" OR "gut microbiota") AND ("neoplasm progression" OR "cancer progression") AND ("therapeutics" OR "chemotherapy" OR "radiation therapy" OR "surgery" OR "immunotherapy")
Google Scholar	("colorectal cancer" AND "gut microbiota" AND "neoplasm progression" OR "cancer progression" AND ("therapeutics" OR "chemotherapy" OR "radiation therapy" OR "surgery" OR "immunotherapy"))

MeSH – Medical Subject Headings; MH – searches the exact CINAHL Plus Subject Heading, searching both major and minor headings; TI – searches the Title field; AB – searches the Abstract field.

the type of treatment modalities examined; the outcome measures (cancer progression and survival rates); and the main findings.

To assess the agreement between the 2 reviewers during the data extraction process, the inter-rater reliability was calculated using Cohen's kappa statistic. The values of the kappa statistic range from –1 to 1, with 1 indicating perfect agreement, 0 indicating no more agreement than would be expected by chance, and –1 indicating total disagreement. The kappa statistic was found to be 0.85 in this review, indicating a high level of agreement between the 2 reviewers. The high level of agreement reinforced the robustness and reliability of the data extraction process.

Bias assessment

The Newcastle–Ottawa Scale (NOS)[18] was used for the assessment of the quality of non-randomized studies, as illustrated in Fig. 2.

Results

Study selection process

A total of 471 records were initially identified from various databases, while no records were found in the registers. Prior to the screening process, a number of records were removed due to the exclusion criteria: 69 were review articles; 78 were case reports or editorials; and 31 were not written in English.

Additionally, 62 records were excluded due to the absence of a full-text version, and 49 duplicate records were removed, leaving 293 records for screening. Of these, 182 reports were sought for retrieval, although 36 could not be retrieved. This resulted in a total of 146 reports being assessed for eligibility. Further exclusions were made on the grounds that 51 reports did not respond to the PECO approach or were considered to be off-topic. Following a rigorous screening and evaluation process, 7 studies were included in the review for further synthesis.[19–25]

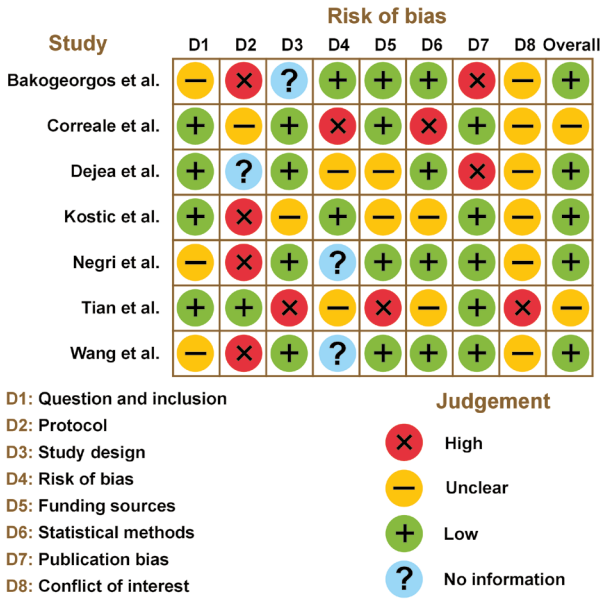

Fig. 2. Evaluation of bias in the selected papers using the Newcastle–Ottawa Scale (NOS)[18]

Demographic characteristics

Table 2 presents the papers selected for inclusion in this review. Collectively, these studies highlight the microbiological role of cancer progression and its correlation with the gut microbiome. The papers exhibited variable sample sizes, ranging from 6 to 120. Several microbiological parameters were assessed in relation to CRC and its treatment.[19–25] These parameters included the details of treatment delivery and chemotherapy toxicity and efficacy,[19] the immunobiological activity of chemoimmunotherapy regimens,[20] and the presence of bacterial biofilms and oncotoxin-encoding genes in patient samples.[21] Some studies focused on particular microbial entities, such as the enrichment of *Fusobacterium* spp. in human colon and stool samples.[22] Other studies examined genetic factors, including FcγR polymorphisms and their role in cetuximab-mediated antibody-dependent cellular cytotoxicity (ADCC).[23] Additionally, clinical trials evaluating combined treatment strategies for CRC were documented,[24] along with the gut microbiome analysis in the context of CRC treatment.[25]

Overall results

Upon analysis, a significant association was observed between CRC and gut microbiota dysbiosis. Patients with CRC demonstrated consistent alterations in the composition and diversity of their gut microbiota when compared to both the normal gut microbiota group and those undergoing different treatment modalities. Dysbiosis was characterized by changes in the relative abundance of specific microbial taxa, which may be indicative of a distinct microbial profile associated with CRC.

Discussion

The reason for focusing our investigation on the microbiology of CRC was driven by a multitude of compelling factors. The human gut, the primary site of CRC, is home to a complex and diverse microbial ecosystem. The gut microbiota is essential for maintaining the health of the host and has been linked to several pathological conditions, including CRC.[9] The intricate relationship between the gut microbiota and CRC represents a promising field for exploration. The existing evidence indicates that dysbiosis of the gut microbiota, defined as an imbalance in

Table 2. Characteristics of the selected studies

Study name	Sample size, *n*	Microbiological parameters assessed	Outcomes
Bakogeorgos et al. 2013[19]	94	treatment delivery (type, dose intensity, relative dose intensity, duration); chemotherapy toxicity and efficacy (ORR, OS, PFS)	• elderly patients were more likely to receive single-agent chemotherapy • no difference was observed in the rate of severe toxicities • ORR, PFS and OS were similar between the 2 groups
Correale et al. 2014[20]	120	immunobiological activity and antitumor efficacy of the GOLFIG chemoimmunotherapy regimen	• GOLFIG regimen showed superior efficacy over FOLFOX in terms of the PFS and the response rate, with a trend towards prolonged survival • patients in the experimental arm showed a higher incidence of non-neutropenic fever, autoimmunity signs and changes in immune cell counts
Dejea et al. 2018[21]	6	presence of bacterial biofilms in the colonic mucosa of FAP patients; enrichment of oncotoxin-encoding genes	• tumorigenic bacteria were associated with the development of early colon neoplasia • co-colonization of *Escherichia coli* and *Bacteroides fragilis* was found to accelerate tumor onset • *E. coli* and *B. fragilis* formed biofilms in the colonic mucosa, with a notable enrichment of oncotoxin-encoding genes
Kostic et al. 2013[22]	61 intestinal stool samples	enrichment of *Fusobacterium* spp. in human colonic adenomas and stool samples	• enrichment of *Fusobacterium* spp. in human colonic adenomas has been observed to result in increased tumor multiplicity • recruitment of tumor-infiltrating immune cells has been demonstrated to create a pro-inflammatory environment that promotes the progression of colorectal neoplasia
Negri et al. 2014[23]	86	FcγR polymorphisms and cetuximab-mediated ADCC	• peripheral blood mononuclear cells harboring the FcγRIIIa 158 V/V genotype had significantly higher cetuximab-mediated ADCC • no correlation was identified between FcγR polymorphisms and the response rate or time to progression following cetuximab-based therapy
Tian et al. 2023[24]	37	clinical trial of combined PD-1, BRAF and MEK inhibition with spartalizumab, dabrafenib and trametinib in patients with BRAFV600E CRC	• study met its primary endpoint, demonstrating a confirmed response rate (24.3% in all patients; 25% in microsatellite stable patients) and durability that were favorable compared to historical controls of BRAF-targeted combinations alone • single-cell RNA sequencing revealed greater induction of tumor cell-intrinsic immune programs and more complete MAPK inhibition in patients with a better clinical outcome
Wang et al. 2021[25]	42	phase Ib/II study of regorafenib in combination with toripalimab for CRC; gut microbiome analysis of the baseline fecal samples	• ORR was 15.2% and the disease control rate was 36.4% in evaluable patients • median PFS and the median OS were 2.1 months and 15.5 months, respectively • patients with liver metastases exhibited a lower ORR than those without liver metastases • gut microbiome analysis revealed significantly increased relative abundance and a positive detection rate of *Fusobacterium* spp. in non-responders compared to responders • patients with high-abundance *Fusobacterium* spp. demonstrated a shorter PFS than those with low-abundance *Fusobacterium* spp.

PFS – progression-free survival; OS – overall survival; FAP – familial adenomatous polyposis; ADCC – antibody-dependent cellular cytotoxicity; PD-1 – programmed cell death protein-1; CRC – colorectal cancer; ORR – overall response rate.

the regular microbial community, may contribute to the onset and progression of CRC.[8] Nevertheless, the precise mechanisms and extent of this involvement remain unclear. Moreover, CRC is among the leading causes of cancer-related deaths globally.[1,4] A deeper understanding of the role of the microbiota in CRC could provide insights into the pathogenesis of the disease, prognosis and potential treatment options.

The progression of cancer is a complex process influenced by a multitude of factors, including genetic, environmental and lifestyle aspects.[1] Recently, there has been a growing recognition of the role of microbiological elements, specifically the role of the microbiota, in this process. The microbiota, particularly the gut microbiota, plays a critical role in maintaining the balance within the human body. Disruptions to this balance can contribute to disease, including cancer.[4,5] Dysbiosis, or an imbalance in the composition of the microbiota, can lead to an environment that promotes cancer progression. For instance, certain bacteria may produce toxins that damage DNA and promote cellular mutations, leading to cancer. Additionally, other bacteria may contribute to the development of cancerous changes in cells by promoting inflammation.[10]

In the study by Bakogeorgos et al., it was observed that elderly patients tended to receive single-agent chemotherapy more frequently than other known interventions.[19] The rate of severe toxicities did not differ significantly between the 2 groups. Furthermore, the overall response rate (ORR), progression-free survival (PFS) and overall survival (OS) were similar across the groups, suggesting comparable efficacy regardless of age and treatment intensity.[19] The study by Correale et al. demonstrated the superior effectiveness of the GOLFIG regimen over FOLFOX, as evidenced by an improved PFS and response rate.[20] However, the experimental arm displayed a higher

incidence of non-neutropenic fever, signs of autoimmunity and changes in immune cell counts, indicating an elevated immune response.[20]

As evidenced by the findings of Dejea et al., tumorigenic bacteria, specifically *Escherichia coli* and *Bacteroides fragilis*, have been associated with the early stages of colon neoplasia.[21] The co-colonization of these bacteria was demonstrated to accelerate tumor onset, with the formation of biofilms in the colonic mucosa and a notable enrichment of oncotoxin-encoding genes.[21] In the study by Kostic et al., *Fusobacterium* spp. was found to be enriched in human colonic adenomas and was associated with increased tumor multiplicity.[22] This enrichment was demonstrated to promote the recruitment of tumor-infiltrating immune cells, thereby creating a pro-inflammatory environment that is conducive to the progression of colorectal neoplasia.[22] Negri et al. observed that peripheral blood mononuclear cells harboring the FcγRIIIa 158 V/V genotype exhibited significantly higher cetuximab-mediated ADCC.[23] However, no correlation was observed between FcγR polymorphisms and the response rate or time to progression following cetuximab-based therapy. This finding suggests that other factors may influence the treatment response.[23]

In the study by Tian et al., the primary endpoint was met, with a confirmed response rate that was favorable relative to historical controls of BRAF-targeted combinations alone.[24] Notably, single-cell RNA sequencing showed a greater induction of tumor cell-intrinsic immune programs and more complete MAPK inhibition in patients with a better clinical outcome.[24] Wang et al. reported an ORR of 15.2% and a disease control rate of 36.4% in evaluable patients.[25] Patients with liver metastases had a lower ORR than those without. Furthermore, the examination of the gut microbiome revealed a significantly increased relative abundance and positive detection rate of *Fusobacterium* spp. in non-responders when compared to responders. Patients with high levels of *Fusobacterium* spp. exhibited a shorter PFS than those with low levels, underlining the potential influence of the microbiota on treatment outcomes.

The findings from our analysis are in close alignment with the observations reported by Wong and Yu and Villéger et al., further emphasizing the potential of the gut microbiota as an influential factor in CRC treatment and prognosis.[26,27] Similar to our findings, the review by Wong and Yu highlighted the role of *Fusobacterium nucleatum*, *E. coli* and *B. fragilis*, and underscored the significance of these bacteria in colorectal carcinogenesis and treatment outcomes.[26] Furthermore, their review emphasized the potential clinical applications of gut microbiota analysis, including its use as a screening, prognostic or predictive biomarker, as well as the possibility of modulating the microbiota for CRC prevention or treatment. These propositions are in accordance with the conclusions drawn from our study, which underscores the potential for integrating microbiota considerations into cancer treatment strategies. In comparison, Villéger et al. focused on the potential of microbial markers for non-invasive early diagnosis and/or prognostic assessment of CRC and advanced adenomas.[27] While our analysis did not explore this aspect in detail, the observed disruption in the gut microbiota balance and the alteration in the fecal metabolome of CRC patients resonates with our findings on the role of the gut microbiota in influencing cancer treatment outcomes. Furthermore, Villéger et al. proposed the use of microbial variation markers as predictors of treatment response,[27] which is consistent with our study's findings on the potential influence of the gut microbiota on treatment effectiveness.

However, while both studies extensively discussed the potential use of the gut microbiota for CRC screening and prognosis,[26,27] our study additionally highlighted the potential role of genetic factors, such as specific genetic polymorphisms, in modulating treatment efficacy. This underscores the need for a holistic approach that considers both the microbiota and genetic factors in CRC management.

Certain bacteria can cause chronic inflammation, which has been associated with various types of cancer. A persistent cycle of cell damage and repair resulting from chronic inflammation increases the likelihood of DNA replication errors and, consequently, mutations. For instance, chronic inflammation caused by the *Helicobacter pylori* infection is known to increase the risk of gastric cancer.[4] Arthur et al. demonstrated that inflammation increases the abundance of *E. coli* and alters its genes, potentially promoting tumor development.[2] Rhee et al. showed that the *B. fragilis* toxin induces colitis and histopathological changes in mice, and suggested that it may lead to subclinical colitis in humans.[4] Yu and Schwabe claimed that the gut microbiota may promote the progression of liver disease and hepatocellular carcinoma via mechanisms such as gut leakiness and bacterial dysbiosis.[9] Wu et al. discovered that *B. fragilis* triggers colitis and induces tumors via a STAT3- and TH17-dependent pathway, thereby providing insights into colon carcinogenesis.[5] Ma et al. demonstrated that the gut microbiota can impact the effectiveness of cancer drugs, potentially affecting chemotherapy and immunotherapy outcomes.[3]

The microbiota can modulate the body's immune response, which plays a vital role in identifying and eliminating cancer cells.[28] Some bacteria may suppress the immune response, allowing cancer cells to evade detection and destruction by the immune system.[29–31] Other bacteria may enhance immune responses, potentially leading to an overactive immune system and chronic inflammation, both of which may contribute to the progression of cancer.[32] Certain bacteria can cause metabolic changes that promote cancer.[33] For instance, some gut bacteria are capable of metabolizing dietary components into carcinogenic compounds. An example is the conversion

of dietary choline and carnitine into trimethylamine by gut bacteria, which is further converted into a proatherogenic compound, trimethylamine N-oxide (TMAO), in the liver.[33–35]

In certain cases, bacteria or their products may translocate from the gut to other parts of the body, leading to inflammation and potentially promoting cancer.[36] This phenomenon is often observed in the context of leaky gut syndrome, where the integrity of the intestinal barrier is compromised.[4] Microbes can also influence the effectiveness of cancer therapies. Some bacteria are capable of metabolizing chemotherapeutic agents, reducing their effectiveness.[37–40]

Limitations

When interpreting the findings of this study, several limitations must be acknowledged. Firstly, the heterogeneity among the studies included in the analysis represents a significant constraint. The analyzed studies employed different methodologies, treatment regimens and patient cohorts, which inherently introduce variability in the results and limit the ability to draw definitive conclusions. The disparities in the sample size and the lack of uniformity in the assessment parameters across the studies may have influenced the outcomes and subsequent interpretations. Secondly, although the study highlighted the role of specific gut microbiota, including *Fusobacterium* spp., *E. coli* and *B. fragilis*, in influencing cancer treatment outcomes, the complexity of the gut microbiota extends beyond these identified species. The gut microbiome is a complex ecosystem comprising a vast array of microbial species, and the collective interactions and functions of these species could influence therapy response. However, this study exhaustively explored this topic. Moreover, the role of genetic polymorphisms was evaluated in a limited context, focusing on FcγR polymorphisms and cetuximab-mediated ADCC. A more expansive range of genetic factors may exert an influence on the response to various cancer treatments, which were not addressed in this study. Lastly, the study focused primarily on CRC, which may limit the generalizability of the findings to other types of cancer. The relationship between the gut microbiota, genetic factors and treatment outcomes may vary across different types of cancer due to the specific genetic and microenvironmental characteristics of each cancer type.

Conclusions

The observed alterations in microbial composition indicate a potential association between the gut microbiota and the progression of CRC. This was particularly evident in the modulation of drug efficacy through a multitude of mechanisms, including direct metabolism of the therapeutic agents, immunomodulation, bacterial translocation, enzymatic degradation, reduction in microbiota diversity, and ecological variability. This finding emphasizes the importance for further investigation into the role of the gut microbiota in CRC pathogenesis. Such research could facilitate the development of targeted interventions aimed at modulating the microbiota to influence disease progression and treatment outcomes in these patients. Further research is warranted to elucidate the mechanisms underlying this association and to explore the therapeutic implications of modulating the gut microbiota in the context of CRC management. However, it was also inferred that despite the compelling evidence indicating the role of the gut microbiota in oncogenesis and cancer treatment, numerous intricacies remain to be elucidated. A deeper understanding of the complex interactions between the host, the microbiota and cancer is essential to fully recognize the therapeutic potential of modulating the gut microbiota. This underscores the necessity for further research employing robust experimental designs and longitudinal studies to elucidate the temporal and causal relationships involved.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Giuseppe Minervini https://orcid.org/0000-0002-8309-1272
Sahana Shivakumar https://orcid.org/0000-0001-9564-1406
Vincenzo Ronsivalle https://orcid.org/0000-0003-3059-4981
Rocco Franco https://orcid.org/0000-0002-2398-7843
Marco Cicciù https://orcid.org/0000-0003-2311-9728
Maria Maddalena Marrapodi https://orcid.org/0000-0002-9494-6942

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Review

Differential diagnosis and treatment protocols for desquamative gingivitis: A systematic review

Hamed Mortazavi[1,A,E,F], Reza Amid[2,A,E,F], Anahita Moscowchi[2,A,C,E,F], Amir-Ali Yousefi-Koma[3,A–E], Hannaneh Yousefi-Koma[4,C–E]

[1] Department of Oral Medicine, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Periodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3] Dentofacial Deformities Research Center, Research Institute for Dental Sciences, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4] Tehran Heart Center, School of Medicine, Tehran University of Medical Sciences, Iran

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):747–758

Address for correspondence
Amir-Ali Yousefi-Koma
E-mail: amiraliykoma@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on August 7, 2022
Reviewed on October 19, 2022
Accepted on October 31, 2022

Published online on October 31, 2024

Abstract

The occurrence of desquamation, shedding and erythema on marginal and attached gingiva is described as desquamative gingivitis (DG). Various autoimmune/dermatological disorders cause DG.

The aim of the present systematic review was to gather information on all possible kinds of treatment for DG, based on specific DG diagnoses.

The review was organized following the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) protocol. An electronic search was conducted in the PubMed, Scopus and Google Scholar databases, up to April 2022. Reviews, letters and studies with less than 2 participants were excluded.

Fifteen publications matched the eligibility criteria: 6 case series; 5 clinical trials; 3 randomized clinical trials (RCTs), and 1 cohort study. A total of 330 patients were enrolled, mostly women (81.52%), with an average age of 57.6 years. Diagnostic characteristics corresponded to oral lichen planus (OLP) ($n = 249$), mucous membrane pemphigoid (MMP) ($n = 30$), pemphigus vulgaris (PV) ($n = 19$), plasma cell gingivitis (PCG) ($n = 4$), erythema multiforme (EM) ($n = 1$), and non-specified diseases (NSD) ($n = 27$). Oral lichen planus and MMP were eliminated using oral hygiene instructions with topical clobetasol and/or doxycycline monohydrate. Pemphigus vulgaris, PCG and EM were treated with topical clobetasol.

To conclude, each DG case requires personalized treatment, depending on the diagnosis.

Keywords: treatment, oral lichen planus, mucous membrane pemphigoid, pemphigus vulgaris, desquamative gingivitis

Cite as
Mortazavi H, Amid R, Moscowchi A, Yousefi-Koma AA, Yousefi-Koma H. Differential diagnosis and treatment protocols for desquamative gingivitis: A systematic review. *Dent Med Probl.* 2024;61(5):747–758. doi:10.17219/dmp/156167

DOI
10.17219/dmp/156167

Introduction

Desquamative gingivitis (DG) is a painful condition with the erythema, bleeding, shedding, and desquamation of gingiva. However, there is no consensus regarding its treatment protocol.

The term "desquamative gingivitis" was initially proposed by Prinz back in 1932 to designate the presence of desquamation, erythema, erosion, and blisters in both marginal and attached gingiva, and even in oral mucosa.[1] Erosion and shedding in DG are completely different than plaque-induced inflammation.[2,3]

Desquamative gingivitis is a distinctive clinical representation of either hypersensitivity reactions to some allergens or a variety of autoimmune diseases. Some antigens incorporated in mouth rinses, toothpastes, chewing gums, and foods can cause hypersensitivity reactions in patients, showcased in the form of DG.[4] In addition, oral lichen planus (OLP), mucous membrane pemphigoid (MMP), pemphigus vulgaris (PV), plasma cell gingivitis (PCG), erythema multiforme (EM), bullous pemphigoid (BP), systemic lupus erythematous (SLE), linear IgA diseases, dermatitis herpetiformis (DH), and epidermolysis bullosa (EB) are some of autoimmune diseases that stimulate DG signs and symptoms.[5,6] Based on the current evidence, OLP is the most common cause of DG of an autoimmune origin. Desquamative gingivitis emerges more frequently in older women, especially during menopause. However, it can also appear in men and children.[7]

Desquamative gingivitis presents moderate pain, mainly due to the exposure of the gingival connective tissue. Additionally, plaque-induced marginal inflammation may intensify the pain.[7] In some cases, pain is the first manifestation of DG.[8] Periodontal studies show that the gingivoperiodontal status is much worse in patients with DG associated with MMP than in the control group.[9–11] Patients with DG related to OLP and PV exhibit deeper pockets and higher levels of clinical attachment loss (CAL).[12,13]

Some evidence suggests that DG plays a potentially significant role in increasing the long-term risk of periodontal tissue breakdown.[11] As DG is a clinical term representing an oral expression of several different mucocutaneous diseases, its treatment is widely diversified.[2–4] Any treatment protocol must be attempted with the purpose of reducing and controlling the signs and symptoms of DG with minimum side effects.[5] Inappropriate home oral hygiene worsens the gingival status of DG patients.[14] Therefore, some authors suggest non-surgical periodontal procedures as possible treatment for DG.[15] Moreover, specific therapies have been introduced, focusing on the general manifestations of the diseases related to the pathogenesis of DG. Topical treatment, mainly with corticosteroids, has also been prescribed in different forms and dosages. Corticosteroids and other immunosuppressants, along with broad-spectrum antibiotics, are among the various drugs administered systematically for DG.[16,17] In addition to corticosteroids, a variety of pharmacotherapies, low-level laser therapies, chlorhexidine, hyaluronic acid, propolis extract, benzydamine hydrochloride mouthwash, and different supplements (e.g., melatonin, vitamin C and vitamin D) have also been assessed for the treatment of DG.[18–23]

To the best of the authors' knowledge, there are no comprehensive guidelines or instructions for treating DG. Two systematic reviews have been conducted, focusing on the management of DG.[24,25] However, many more studies, procedures and protocols could be included. Therefore, the aim of this systematic review was to gather all clinical studies that used any kind of treatment for DG in order to precisely conclude which therapeutic procedures for DG are the safest and most efficient, with the fewest side effects.

Methods

This study has been prepared and organized according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines.[26]

Information sources, the search strategy and study selection

In this review, an electronic search was conducted employing the PubMed, Scopus and Google Scholar databases. Our search was limited to English-language studies published between January 1965 and April 2022. The electronic search was complemented with a manual search of the reference lists of all relevant articles and previous systematic reviews. The following search method was used to find relevant studies in the abovementioned databases: (desquamative gingivitis) AND (treatment OR therapeutics). Eligibility checking, followed by data extraction, was performed independently by 2 reviewers. The inclusion and exclusion criteria of the review were initially applied through the screening of titles and abstracts. Any type of human clinical studies focusing on the treatment of DG (e.g., case series, case reports, randomized controlled trials (RCTs), and cohort studies) were included. Systematic and narrative reviews, letters, comments, studies with fewer than 2 participants, in vitro studies, and animal in vivo/ex vivo studies were all excluded from this review. Duplicated studies were recognized and removed. Later, the full texts of the related studies were reviewed for eligibility. In any case of disagreement, a third reviewer was consulted.

Data collection

Several data items were used for clinical studies: (1) demographics (gender and age) of the included participants; (2) type of study; (3) number of cases; (4) diagnosis of DG

– the relation or association of DG with autoimmune mucocutaneous diseases (OLP, MMP, PV, PCG, and EM) in each case; (5) duration of DG in patients; (6) distribution of DG lesions; (7) previous treatment; (8) treatment protocols, procedures, approaches, and plans; (9) study variables; (10) evaluation period; and (11) outcomes.

Quality assessment

A Cochrane risk of bias assessment tool (RoB 2) was applied for both randomized and non-randomized studies to assess the risk of bias. Each study was analyzed with the prefabricated questions created by RoB 2.

Synthesis methods

Taking into account the data extracted from the included studies, it appeared that the methods and protocols used for the treatment of DG were widely diversified. Hence, it was not possible to perform a meta-analysis. The descriptive analysis of the data extracted from clinical studies, along with a narrative and graphical synthesis, were performed.

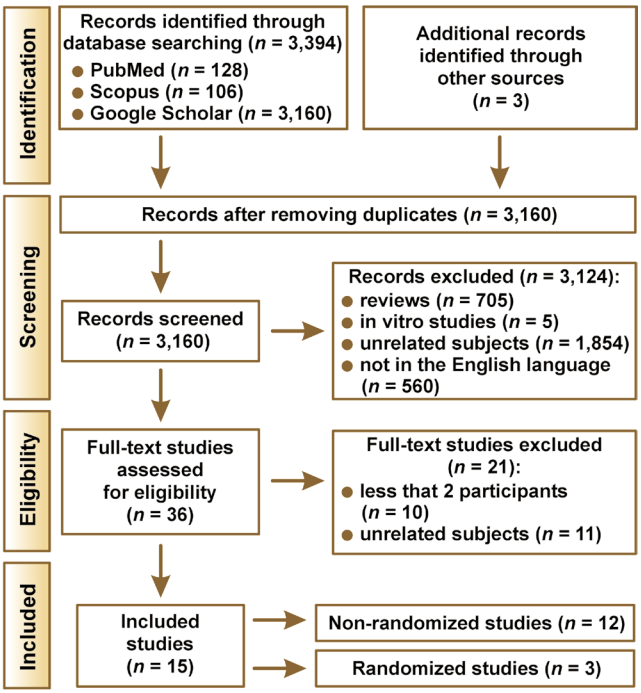

Fig. 1. Study selection process

Results

Study selection

Figure 1 displays the diagram of study selection. The initial search yielded 3,397 publications, from which 3,124 irrelevant articles were excluded based on their titles and abstracts. The remaining 36 studies were assessed for eligibility based on the inclusion criteria and the full text of each article was retrieved for further evaluation. After a detailed review, 12 records were retained for data extraction. Three additional records were identified by reading the reference lists in each included study. The selected studies were published between 1987 and 2019. The studies came from Italy (n = 5),[15,27–30] Brazil (n = 3),[31–33] Norway (n = 2),[34,35] Spain (n = 1),[36] Denmark (n = 1),[37] Switzerland (n = 1),[38] Scotland (n = 1),[39] and the United Kingdom (n = 1).[16]

Results of individual studies

Demographics for all the patients included in the studies are displayed in Table 1. The study type, the distribution of DG lesions, the duration of DG in patients, the previous treatment of DG in patients prior to the study, the treatment procedures and approaches used in the studies, the study variables, the evaluation period, and outcomes for all of the 15 included studies are detailed in Table 2.

Study characteristics

Study design

As specified by the methodological design, different types of studies were as follows: case series (prospective) (n = 6)[28,29,35,37–39]; clinical trials (n = 5)[15,31,33,34,36]; RCTs (n = 3)[16,27,30]; and a cohort study (n = 1).[32]

Demographics

The final sample, demographic characteristics and diagnostic characteristics of the enrolled patients (after the elimination of patients who did not complete the treatment and/or follow-ups) are displayed in Table 1. The total number of analyzed subjects was 330 (81.52% women). The age of participants ranged from 21 to 89 years, while the average age was 57.6 years.

Diagnosis

Out of the 15 included studies, in only one RCT, the authors did not declare the diagnosis of DG in their participants (n = 24).[30] Rønbeck et al. also had 3 cases with non-specified diseases (NSD).[34] However, in the remaining 13 studies, the diagnoses of diseases in participants corresponded to 249 OLP cases, 30 MMP cases, 19 PV cases, 4 PCG cases, and 1 EM case.

Distribution

Some studies reported the exact sites affected by DG,[15,27,28,30,33,37–39], while others did not declare the exact gingival sites affected by DG in their participants.[16,29,31,32,34–36]

Duration

The duration of DG was declared in 5 studies, ranging from 1 to 40 years.[32,34,35,37,38]

Previous treatment

Previous treatment was not mentioned in 4 studies.[27–30] Some participants were not prescribed any systemic and/or topical non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids or antibiotics for 6 weeks,[35] 2 months[31,34] to 3 months[15,31,36,37] prior to the study. Other patients[16,32–34,38,39] received treatment prior to the study, detailed in Table 2.

Treatment procedures/approaches

There was a variety of different methods, therapeutics and protocols used for the treatment of DG, such as: oral hygiene protocols[28,29,31,36,37]; sonic and manual (soft-bristle) tooth brushing and interdental cleaning devices[15,16,27,36]; plaque control[16,31]; scaling and root planing (SRP)[15,28,36]; alcohol-free chlorhexidine digluconate[15]; topical fertomcidina-U[29]; topical corticosteroids (clobetasol)[15,30,32,36]; immunosuppressive agents (topical tacrolimus)[30]; topical fusidic acid[33]; doxycycline monohydrate[34,35]; topical steroids maintained by self-retaining acrylic labial veneers[39]; and rituximab.[38] Evaluation periods after the completion of treatment varied from 2 weeks[32] to 2 years.[38]

Table 1. Demographic and diagnostic characteristics of the included studies

Study	Gender *n*			Age [years]		Mucocutaneous diseases					
	total	M	F	*M* (±*SD*)	range	OLP	MMP	PV	PCG	EM	NSD
Guiglia et al.[15] 2007	30	5	25	61.37 ±11.22	41–82	30	–	–	–	–	–
Stone et al.[16] 2015	79	15	64	61.4	NS	79	–	–	–	–	–
Bianco et al.[27] 2019	32	12	20	cases: 61.0 ±9.3 controls: 65.4 ±11.1	51–76	32	–	–	–	–	–
Arduino et al.[28] 2012	12	–	12	59.5 ±14.5	NS	–	12	–	–	–	–
Carcieri et al.[29] 2016	20	3	17	56.95 ±16.75	NS	9	1	6	4	–	–
Corrocher et al.[30] 2006	24	6	18	cases: 47.3 controls: 54.6	21–65	–	–	–	–	–	24
Salgado et al.[31] 2013	20	2	18	55.9	39–75	20	–	–	–	–	–
Fragoso Motta et al.[32] 2009	22	5	17	50.6 ±16.4	25–78	9	5	8	–	–	–
Manzolli Leite et al.[33] 2015	15	2	13	55.2	39–76	7	3	5	–	–	–
Rønbeck et al.[34] 1990	14	3	11	59.9	48–75	6	4	–	–	1	3
Lind and Hurlen[35] 1988	3	1	2	60	58–62	1	2	–	–	–	–
López-Jornet and Camacho-Alonso[36] 2010	40	5	35	57.62 ±12.33	33–89	40	–	–	–	–	–
Holmstrup et al.[37] 1990	11	–	11	59.8	43–76	11	–	–	–	–	–
Haefliger et al.[38] 2016	3	2	1	60	50–70	–	3	–	–	–	–
Wray and McCord[39] 1987	5	–	5	NS	NS	5	–	–	–	–	–
Total	330	61	269	57.6	21–89	249	30	19	4	1	27

M – male; F – female; *M* – mean; *SD* – standard deviation; OLP – oral lichen planus; MMP – mucous membrane pemphigoid; PV – pemphigus vulgaris; PCG – plasma cell gingivitis; EM – erythema multiforme; NSD – non-specified disease; NS – not specified.

Table 2. Study type, distribution of desquamative gingivitis (DG) lesions, duration of DG, previous treatment, treatment procedures/approaches, study variables, evaluation period, and outcomes for the included clinical studies

Study	Study type	Distribution of DG lesions	Duration of DG	Previous treatment	Treatment procedures/approaches	Study variables	Evaluation period	Outcomes
Guiglia et al.[15] 2007	single-blind, open clinical trial	marginal gingiva lesions and vestibular lesions	NS	none had received systemic antibiotics and/or corticosteroids 3 months prior to the study	supragingival and subgingival scaling, polishing, a soft-bristle toothbrush (modified Bass technique), alcohol-free chlorhexidine digluconate mouthwash (0.2%, 10 mL, twice a day, for 1 week), topical clobetasol (once, twice or thrice a day, for 5 min), along with follow-ups at the end of each month	– PI – BoP	3 months	PI and BoP were significantly reduced in all sites
Stone et al.[16] 2015	parallel-group, longitudinal RCT	NS	NS	topical steroids (n = 23), systemic steroids (prednisolone) (n = 3)	structured plaque control: powered toothbrushes and interdental cleaning devices	– OHIP – mucosal diseases – PI – VAS	4 and 20 weeks	a statistically significant decrease in mucosal diseases, patients' pain and PI, along with improvement in OHIP
Bianco et al.[27] 2019	parallel-group, examiner-blinded RCT	marginal and attached gingiva lesions, without other mucosal lesions	NS	NS	sonic and manual (soft-bristle) tooth brushing (twice daily) cases (n = 16): sonic controls (n = 16): manual	– MMP-1 and MMP-9 levels in GCF – PI – BoP – VAS	8 weeks	significant periodontal improvement in both groups; significant decreases in the MMP-1 and MMP-9 levels in GCF in the case group
Arduino et al.[28] 2012	pilot study, prospective case series	attached gingiva lesions and palatal lesions	NS	NS	oral hygiene instructions and non-surgical periodontal therapy (SRP and tooth polishing)	– FMPS – FMBS – VAS	5 weeks	a statistically significant reduction in FMPS, FMBS and patients' painful symptoms
Carcieri et al.[29] 2016	prospective case series	gingival lesions (authors did not describe the exact distribution of lesions)	NS	NS	oral hygiene protocol combined with topical fertomcidina-U; fertomcidina-U was used at each session (3 weekly sessions for 12 months), impregnated gauze (5 mL) was applied for 15 min on each jaw	– FMPS – FMBS – PPD – VAS	2 months	a significant reduction in FMPS, FMBS and reported pain, along with gingival improvement
Corrocher et al.[30] 2006	double-blind RCT	marginal and attached gingiva lesions	NS	NS	tacrolimus (0.1%, 2 mL) or clobetasol (0.5%, 2 mL) once a day, for 4 weeks; medications were in identical packages to provide blinding	– erythema – desquamation	4, 6 and 8 weeks	tacrolimus resulted in a significant reduction in signs, performing much better than clobetasol tacrolimus group: 11 patients observed the remission of desquamation and/or erythema at weeks 4 and 6, 6 patients reported a mild oral burning sensation clobetasol group: none of the patients attained the remission of either sign, 6 patients reported mild mouth dryness
Salgado et al.[31] 2013	short-term study	NS	NS	none had received topical steroids and antibiotics, and/or NSAIDs 2 and 3 months prior to the study, respectively	plaque control: oral prophylaxis followed by weekly professional plaque removal	– VAS – clinical features	30 days	improvement in clinical features, along with a significant decrease in painful symptoms

Study	Study type	Distribution of DG lesions	Duration of DG	Previous treatment	Treatment procedures/approaches	Study variables	Evaluation period	Outcomes
Fragoso Motta et al.[32] 2009	double-blind, crossover, placebo-controlled clinical trial	chronic gingival lesions (authors did not describe the exact distribution of lesions)	group 1 (5 F): 8.2 ±3.4 years group 2 (5 M, 12 F): 5.6 ±4.0 years	group 1: systemic azathioprine and/or prednisone, for at least 6 months prior to the study group 2: no systemic treatment	0.05% clobetasol propionate ointment or placebo in trays (thrice a day for 2 weeks, and once a day in the 3rd week): each patient using both clobetasol and placebo blindsided with a 2-week gap	– erythema – atrophy – VAS – clinical features	2-week intervals	overall, there was no significant difference between the effects of clobetasol and placebo; – signs clobetasol: improvement of signs in 17 patients, while worsening in the rest placebo: improvement of signs in 18 patients, while worsening in the rest – symptoms clobetasol: worsening in 1 patient, no response in 7, partial improvement in 12, and absolute improvement in 2 placebo: worsening in 2 patients, no response in 12 and partial improvement in 8
Manzolli Leite et al.[33] 2015	controlled clinical trial	marginal and attached gingiva lesions (mostly of the maxilla), without destructive periodontal diseases (PD ≥ 5 mm in combination with CAL ≥ 2 mm)	NS	after the development of lesions, all changed their toothbrushes to extra-soft and started using kids' toothpaste corticosteroids	topical 2% fusidic acid ointment (4 times a day, for 6 weeks)	– impact on daily activities – pain score – lesion size	6 weeks and 12 months	a significant reduction in lesion size, pain intensity and periodicity
Rønbeck et al.[34] 1990	controlled clinical trial	erythema and bullae of gingiva propria (authors did not describe the exact distribution of lesions)	1–40 years (mostly 3 years)	7 patients were using systemic drugs: – insulin ($n = 1$) – sulfonamide ($n = 1$) – thyroxine ($n = 2$) – diuretics ($n = 3$) the use of any NSAIDs and/or corticosteroids was discontinued for at least 2 months prior to the study	doxycycline monohydrate (100 mg, once a day, for 4–11 weeks)	– MIS – Nikolsky sign – alveolitis due to desquamation – bleeding and soreness of gingiva	11 weeks	DG lesions worsened in 3 patients (MIS increased by 1 point), no difference was noticed in 1 patient (no change in MIS) and there was significant improvement in 10 patients (MIS decreased by 1 point ($n = 7$), 2 points ($n = 2$) and 3 points ($n = 1$))

Study	Study type	Distribution of DG lesions	Duration of DG	Previous treatment	Treatment procedures/approaches	Study variables	Evaluation period	Outcomes
Lind and Hurlen[35] 1988	case series	NS	in 2 cases: 3 years in 1 case: 9 years	all kinds of treatment were withdrawn 6 weeks prior to the study: –oral hygiene instructions – topical and systemic corticosteroids – chlorhexidine mouthwash – SRP	doxycycline monohydrate (100 mg, once a day, for 8 weeks)	– erythema – desquamation	8 weeks (2-week intervals)	complete remission in 2 cases, with partial remission in the 3rd case; the recurrence of clinical features developed shortly after the withdrawal of the medication
López-Jornet and Camacho-Alonso[36] 2010	descriptive pre- and post-test clinical study	NS	NS	none had received systemic antibiotics and/or corticosteroids 3 months prior to the study	motivation–behavioral skills protocol (problem identification, confidence, behavioral awareness, and relapse prevention), brushing instructions (Bass method), supragingival scaling, tooth polishing, along with topical corticosteroids (1-minute rinse, thrice a day)	– GI – PEI	4 and 8 weeks	a significant reduction in both GI and PEI in all patients
Holmstrup et al.[37] 1990	case series	vestibular and lingual lesions	diagnosis of OLP: 3–14 years	none had received antibiotics and/or NSAIDs 3 months prior to the study	intensive individual oral hygiene procedures	– MPS – VAS – clinical features	1 year (3-month intervals)	a significant decrease in MPS, along with patients' subjective (pain) and objective (clinical features) improvement
Haefliger et al.[38] 2016	case series	gingival mucosa, marginal and attached gingiva	11, 12 and 30 months	dapsone, prednisolone, doxycycline, mycophenolate mofetil, nicotinamide	rituximab (2 infusions of 1,000 mg, with a 2-week interval)	MAS	6 and 24 months	– 6 months: complete remission in 2 cases, with partial remission in the 3rd case – 24 months complete remission in 1 case after treatment, and complete remission with minimal adjunct therapy (dapsone – 12.5 mg per day and sulfasalazine – 1 g per day)
Wray and McCord[39] 1987	case series	upper and lower anterior marginal and attached gingiva	NS	oral hygiene instructions, chlorhexidine mouthwash (0.2%), twice a day	topical steroids maintained on patients' gingiva and gingival mucosa by self-retaining acrylic labial veneers (heat-cured pink acryl of 1-millimeter thickness)	– erythema – desquamation	6 months	no patient became free of DG lesions; however, both desquamation and erythema were significantly reduced

RCT – randomized clinical trial; PD – pocket depth; CAL – clinical attachment loss; NSAID – non-steroidal anti-inflammatory drug; SRP – scaling and root planing; PI – plaque index; BoP – bleeding on probing; OHIP – oral health impact profile; VAS – visual analog scale; MMP – matrix metalloproteinase; GCF – gingival crevicular fluid; FMPS – full-mouth plaque score; FMBS – full-mouth bleeding score; PPD – probing pocket depth; MIS – mucosal index score; GI – gingival index; PEI – plaque extension index; MPS – mean plaque score; MAS – mucosal activity score; NS – not specified.

Reported outcomes

There were 7 studies that used a visual analog scale (VAS) to analyze pain levels.[16,27–29,31,32,37] In some studies, photography was used to assess the extension of lesions.[15,32,34–36,38,39] A variety of other indices and parameters were also collected: the plaque index (PI)[15,16,27]; bleeding on probing (BoP)[15,27]; the full-mouth plaque score (FMPS)[28,29]; the full-mouth bleeding score (FMBS)[28,29]; the probing pocket depth (PPD)[29]; the gingival index (GI)[36]; the plaque extension index (PEI)[36]; the mean plaque score (MPS)[37]; the mucosal index score (MIS)[34]; the mucosal activity score (MAS)[39]; the bleeding and soreness of gingiva[34]; atrophy[32]; erythema[30,32,35,39]; desquamation[30,34,35,39]; the lesion size[33]; the level of matrix metalloproteinase (MMP) 1 and MMP-9 in gingival crevicular fluid (GCF)[27]; the Nikolsky sign[34]; and the oral health impact profile (OHIP).[16]

Using all of the gathered data on treatment protocols and reported outcomes, a flowchart was designed to summarize different mucocutaneous diseases (Fig. 2). All kinds of treatment are listed in the order of their frequency of use. A code is given to each treatment from "A" to "L" in alphabetical order. From now on, each treatment and its outcomes will be discussed referring only to the codes (A–L) rather than the full names or descriptions. All codes with their complete descriptions are listed below:

A) personal oral hygiene instructions; sonic and manual (soft-bristle) tooth brushing, and interdental cleaning devices;
B) plaque control; SRP and tooth polishing;
C) doxycycline monohydrate; 100 mg, once a day, for 4–11 weeks;
D) alcohol-free chlorhexidine digluconate; 0.2%, 10 mL, twice a day, for 1 week;
E) topical clobetasol; once, twice or thrice a day, for 5 min;
F) clobetasol propionate ointment (0.05%) in trays; thrice a day for 2 weeks and once a day in the 3rd week;
G) topical 2% fusidic acid ointment; 4 times a day, for 6 weeks;
H) topical fertomcidina-U; 15 min on each jaw, 3 weekly sessions for 12 months;
I) topical 0.1% triamcinolone acetonide; 15 mL, 1-minute rinse, thrice a day;
J) rituximab; 2 infusions of 1,000 mg, with a 2-week interval;
K) topical steroids maintained by self-retaining acrylic labial veneers;
L) tacrolimus or clobetasol; 0.1% and 0.5%, respectively, 2 mL, once a day, for 4 weeks.

Quality assessment

The summary of the risk of bias in randomized and non-randomized studies is displayed in Fig. 3 and 4, respectively. All randomized studies had an overall high risk of bias due to an individual high risk of bias in terms of selection,[30] performance,[27] detection,[27,30] attrition,[16] or other bias.[30]

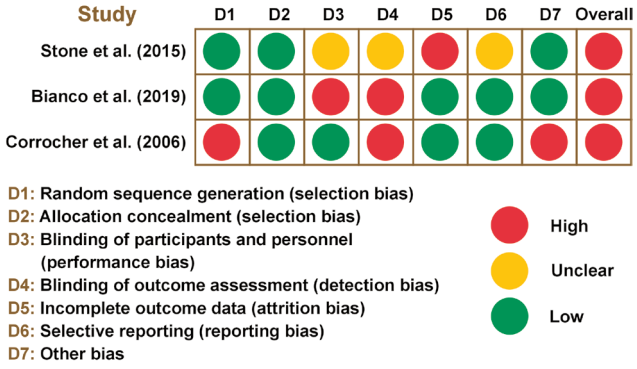

Fig. 3. Risk of bias summary in randomized studies

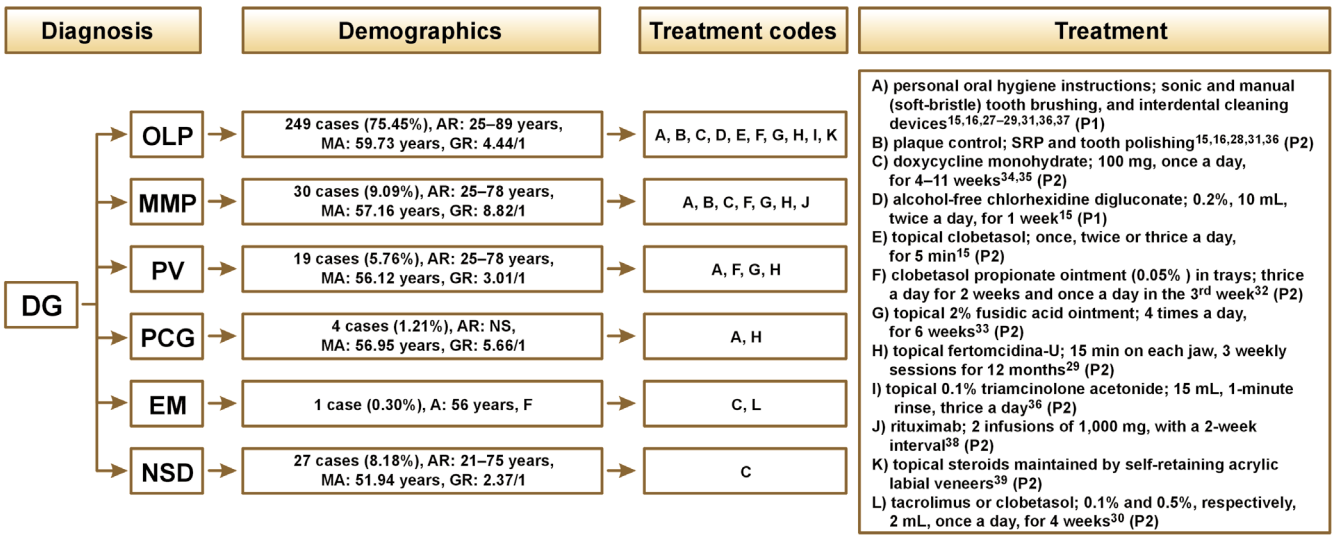

Fig. 2. Summary of demographics and all of the tested kinds of treatment for desquamative gingivitis (DG) in each group of mucocutaneous diseases
A – age; AR – age range; MA – mean age; GR – gender ratio (F/M); P1 – initial (1st) phase of treatment; P2 – 2nd phase of treatment; NS – not specified.

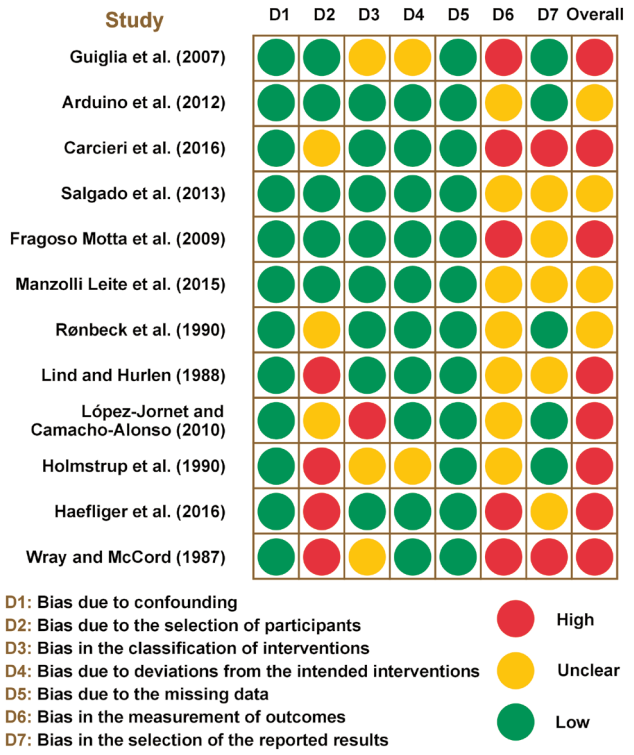

Fig. 4. Risk of bias summary in non-randomized studies

Among non-randomized studies, 8 studies[15,29,32,35–39] had an overall high risk of bias due to an individual high risk of bias in the selection of participants,[35,37–39] the classification of interventions,[36] the measurement of outcomes,[15,29,32,38,39] and the selection of the reported results.[29,39]

Discussion

This study was conducted to systematically review the current therapeutic protocols for DG. All of the different mucocutaneous diseases that can cause DG signs and symptoms, and all of the different kinds of treatment for DG are discussed below.

Mucocutaneous diseases

Oral lichen planus

The term "lichen planus" (LP) is a combination of the Greek word "lichen" and the Latin word "planus", meaning "tree moss" and "flat", respectively.[40] Lichen planus is a chronic immunological, inflammatory disorder affecting hair, skin, nails, and the mucous membrane.[41] Oral lichen planus stimulates intractable oral lesions, exhibits chronicity and rarely undergoes self-remission. Oral lichen planus has various clinical manifestations, with DG appearing in almost 10% of cases.[42,43] It is mediated by macrophages, CD8+ T cells and Langerhans cells. Autoimmune mechanisms precipitate apoptosis, causing cell destruction, followed by characteristic histological changes.[42] Oral lichen planus is frequently correlated with psychosomatic ailments (e.g., stress, anxiety and depression).[44]

More than 75% of the participants enrolled in the 15 included studies were diagnosed with OLP as the mucocutaneous disease responsible for DG signs and symptoms.[15,16,27,29,30,31–37,39] The most common treatment for these patients were A, B and C (Fig. 2). It could be deduced from the extracted data that proper personal oral hygiene instructions combined with the regular SRP and tooth polishing executed by clinicians resulted in a significant reduction of DG signs and symptoms in OLP patients.[15,16,27,31,36,37] Topical 2% fusidic acid ointment (G) showed positive results in the longest evaluation period (12 months).[33]

Mucous membrane pemphigoid

Mucous membrane pemphigus is an autoimmune disease with subepithelial blistering. It is a disease phenotype correlated to one or more disparate anti-basement membrane zone (BMZ) autoantibodies. The hemidesmosome has a variety of components that are targets for the autoantibodies of MMP.[45,46] In a study by Ahmed et al., the age of most MMP patients ranged from 40 to 59 years, and they were mostly women.[47] In another study, 35–48% of all DG cases were diagnosed with MMP.[2] The most common, if not the only, presentation of MMP is DG.[48]

In more than 9% of the participants analyzed in this review, DG was related to MMP.[28,29,32–35,38] The only therapeutic protocol that was used in more than one study for MMP patients was doxycycline monohydrate (C) (Fig. 2), which showed desirable results in both studies.[34,35] Rituximab (J) had desirable outcomes in the longest evaluation period (6 and 24 months).[38]

Pemphigus vulgaris

Pemphigus vulgaris is an autoimmune disease distinguished by suprabasilar separation and by acantholysis in the epithelium of the mucous membrane and/or skin. The key target antigen of PV is a component of the desmosome on keratinocytes.[49] Pemphigus vulgaris can present at any age. However, PV is most commonly diagnosed in middle-aged and elderly patients.[50,51] Pemphigus vulgaris lesions generally start on the buccal mucosa. Gingival involvement in the form of DG can also be observed.[48] If left untreated, PV could be a life-threatening condition.[52] It is responsible for 3–15 % of all DG cases.[2]

The signs and symptoms of DG were caused by PV in almost 6% of all cases in the included studies.[29,32,33] Each of the 3 studies with the PV diagnosis used a different kind of treatment. However, all of them had one aspect in

common – all medications were used topically (F, G, H) (Fig. 2). Topical fusidic acid (G) had positive outcomes through the longest evaluation period.[33]

Plasma cell gingivitis

Plasma cell gingivitis is an unusual benign gingival condition. It is indicated and characterized by the massive and diffuse infiltration of normal plasma cells divided into aggregates by strands of collagen.[53] Plasma cell gingivitis is a hypersensitivity reaction to the antigens found in flavoring agents, chewing gum spices, lozenges, and toothpastes.[54] The condition causes discomfort, bleeding and severe gingival inflammation.[53,55]

Four of the cases in this review were diagnosed with PCG. Topical fertomcidina-U (H) was the only treatment found for DG caused by PCG, resulting in significant improvement in DG signs and symptoms (Fig. 2).[29]

Erythema multiforme

Erythema multiforme is a penetrating, immune-mediated, mucocutaneous condition, mostly induced by herpes simplex virus (HSV) infections and by certain medications.[56] Erythema multiforme lesions initially appear as erythema and edema, and then proceed into superficial erosions and pseudomembrane formations.[57] Oral lesions of EM are mainly characterized by the crusting of lips and by the ulceration of non-keratinized mucosa. Hence, EM rarely causes DG.[58]

Among all of the 330 patients reviewed in this study, only one of them was diagnosed with EM. The treatment used for that patient was doxycycline monohydrate (C), resulting in significant improvement in DG signs and symptoms (Fig. 2).[34]

Non-specified diseases

Corrocher et al. neither declared nor mentioned the diagnosis of their patients (n = 24).[30] Rønbeck et al. had 3 cases that they were unable to diagnose.[34].We gathered these 27 cases and categorized them as DG associated with NSD. Doxycycline monohydrate (C) and tacrolimus or clobetasol (L) were used for these patients, resulting in significant improvement in DG signs and symptoms (Fig. 2).[30,34]

Treatment recommendations

It could be inferred that OLP and MMP are the mucocutaneous diseases responsible for most DG cases reported. It is also conceivable that the majority of DG patients, observed and/or treated, are women in their 6th (or higher) decade of life. Some clinicians believe that any DG patient must undergo an initial phase (P1) of personal oral hygiene instructions (e.g., sonic and manual tooth brushing, interdental cleaning devices, and mouthwash) and professional plaque control (e.g., SRP and tooth polishing). If the results of the 1st phase are underwhelming, they enter the 2nd phase of treatment (P2), with different therapeutics. This assumption could be reliable to some degree; most OLP and MMP cases analyzed in this review used personal oral hygiene instructions (A) and professional plaque control (B), followed by desirable results. However, some DG patients (regardless of their diagnoses) have symptoms regardless of their excellent personal oral hygiene routines and regular periodontal visits. These patients are not proper candidates for the initial phase of DG treatment, and should be introduced to/prescribed with different therapeutics. Antibiotics (e.g., doxycycline monohydrate and fusidic acid), antiseptics (e.g., fertomcidina-U), immunosuppressive agents (e.g., tacrolimus), corticosteroids (e.g., clobetasol), and glucocorticoids (e.g., triamcinolone) can all be used for diminishing DG signs and symptoms. Doxycycline monohydrate and topical clobetasol can both be used for DG patients with OLP and MMP. The published data concerning the treatment of DG is not cohesive and convincing enough to turn into a comprehensive guideline. However, relying on the data extracted from the 15 included studies, a step-by-step protocol was proposed to guide clinicians faced with DG patients.

(1) Differential diagnosis of DG: First, make sure that your patient has DG signs and symptoms, and not the symptoms of other similar gingival conditions.

(2) Diagnosis of mucocutaneous disease: Execute all necessary tests and biopsies (if needed) to diagnose the underlying mucocutaneous disease responsible for DG.

(3) Treatment: If the patient was diagnosed with OLP or MMP, a combination of P1 instructions, along with doxycycline monohydrate (general use) or topical clobetasol could be the safest choice. If the patient is affected by PV, PCG or EM, prescribe topical clobetasol, fusidic acid or fertomcidina-U. (Note: Make sure to ask patients about their systemic condition. In the presence of systemic diseases (e.g., diabetes mellitus, heart diseases), contact their doctors and ask about the indications/contraindications for the suggested therapeutics.)

(4) Follow-ups: Set monthly follow-ups for 1–2 years. Record the data (e.g., VAS, shedding, erythema, bleeding, and clinical scores) to trace the elimination of DG signs and symptoms.

Limitations

Only 7 of the 15 studies used a VAS questionnaire to record pain levels in their participants. Additionally, the studies mostly did not have a lot in common when it came to selecting different indices and parameters for assessment before and after treatment. Hence, comparing the results and outcomes of the studies was not possible.

We only included studies that had at least 2 patients.

By doing this, we had to neglect at least 8 case reports. Therefore, we may have missed out on other possible treatment protocols for DG.

According to our risk of bias assessment (Fig. 3 and 4), our included studies had a moderate-to-high risk of bias on average.

Due to the limited number of studies regarding the treatment of DG, we included all different types of studies (e.g., case–control studies, case series, clinical trials, RCTs, etc.). Evidently, not all of the studies had randomization in their selection of participants, and most of the studies had a moderate-to-high risk of bias.

Suggestions

We strongly encourage clinicians to report the outcomes of different treatment protocols used for DG patients. Any additional study can be an important step toward the establishment of sequential treatment protocols as a guideline for the treatment of DG in the future. The moderate-to-high average risk of bias in the included studies shows that in regard to the treatment of DG, we still need many new studies that are conducted carefully, with maximum randomization, to help us develop a better look at different kinds of treatment and their outcomes, with high rates of reliability.

Conclusions

Each DG case requires personalized treatment, depending on the patient's diagnosis. Most DG cases are caused by OLP and MMP. The safest treatment plan for DG caused by OLP and MMP is personal and professional oral hygiene instructions combined with doxycycline monohydrate or topical clobetasol.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Hamed Mortazavi https://orcid.org/0000-0002-0778-5299
Reza Amid https://orcid.org/0000-0002-8053-3928
Anahita Moscowchi https://orcid.org/0000-0003-1327-5014
Amir-Ali Yousefi-Koma https://orcid.org/0000-0002-0140-8097
Hannaneh Yousefi-Koma https://orcid.org/0000-0001-7498-301X

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Review

Assessing the prevalence and risk of tooth wear in Parkinson's disease: A narrative review

Merel Charlotte Verhoeff[A–F], Peter Wetselaar[A,D–F], Frank Lobbezoo[A,D–F]

Department of Orofacial Pain and Dysfunction, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and the Vrije Universiteit Amsterdam, the Netherlands

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):759–764

Address for correspondence
Merel Charlotte Verhoeff
E-mail: m.c.verhoeff@acta.nl

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on January 12, 2024
Reviewed on January 27, 2024
Accepted on February 8, 2024

Published online on July 19, 2024

Abstract

Tooth wear is an increasingly common problem that affects the quality of life. Although previous research suggested that oral health is adversely affected in individuals with Parkinson's disease (PD) compared to healthy controls, tooth wear has not been extensively studied in this context. Particularly, there is a paucity of data on the prevalence and risk factors associated with tooth wear in PD patients. The aim of this study is to review the current literature on the prevalence and risk factors of tooth wear in PD patients and to propose hypotheses for future research on this topic. A literature search was conducted in PubMed. A total of 4 publications were identified: 1 case report and 3 questionnaire-based studies. These articles suggest that tooth wear is a more significant issue in PD patients than in healthy controls. In addition, potential associations between oral health-related quality of life (OHRQoL), bruxism and temporomandibular disorder (TMD) pain on the one hand, and tooth wear on the other hand, were identified in PD patients. Due to the limited number of articles published on this topic, it is not possible to definitively conclude whether tooth wear is a common problem in PD patients. However, the following hypotheses could be formulated: 1) tooth wear is more prevalent in PD patients than in healthy controls; 2) risk factors for tooth wear observed in healthy individuals are more prevalent among PD patients; and 3) multiple risk factors for tooth wear likely coexist in people with PD, potentially influencing the prevalence and progression of tooth wear in this population.

Keywords: tooth wear, Parkinson's disease, oral health, quality of life

Cite as
Verhoeff MC, Wetselaar P, Lobbezoo F. Assessing the prevalence and risk of tooth wear in Parkinson's disease: A narrative review. *Dent Med Probl.* 2024;61(5):759–764. doi:10.17219/dmp/183842

DOI
10.17219/dmp/183842

Introduction

Tooth wear is a phenomenon that involves the loss of dental hard tissues. The prevalence of tooth wear increases with age, and the severity of tooth wear is progressive.[1] This could result in the acquisition of a severe form of tooth wear with increasing age, which may develop into pathological wear (i.e., moderate tooth wear in combination with 1 or several described signs and symptoms, such as sensitivity).[2] Severe tooth wear is present in 3–17% of the adult population.[3] Although a specific cause may be dominant, the etiology of tooth wear is often multifactorial, and therefore, the individual mechanisms (intrinsic and/or extrinsic mechanical and/or chemical) are rarely present in isolation.[4] This makes tooth wear an interesting phenomenon, yet it is difficult to diagnose and treat. Importantly, the loss of dental hard tissues has a negative impact on the oral health-related quality of life (OHRQoL).[5] Therefore, tooth wear, in all its complexity, is an important component of oral health-related diseases and their management.

Prior research has indicated that OHRQoL is negatively altered in individuals with Parkinson's disease (PD), a neurodegenerative disorder affecting specific parts of the brain, such as the substantia nigra.[6] Symptoms of PD vary between individuals, and both motor and non-motor symptoms can occur, including tremors, a masked face, pain, and cognitive decline.[7] Although younger adults can also be affected by PD, it is more prevalent in the older population.[8] Treatment of PD consists primarily of symptom relief using medications that increase dopamine levels in the structures of the extrapyramidal system, such as levodopa (a precursor of dopamine that can pass the blood–brain barrier). In addition, physiotherapy, speech therapy and brain surgery could also be indicated and helpful in alleviating the symptoms of PD.[9]

A recent study has indicated that oral health, in its broadest sense, is worse in PD patients than in healthy controls.[10] Although tooth wear is an ever-growing problem, there is a paucity of data regarding the prevalence and risk of tooth wear in PD patients. Consequently, the question arises whether tooth wear is a larger problem in PD patients than in their healthy peers, and whether the risk factors for tooth wear, which are known to be present in the general population, are also present in PD patients. Therefore, the aim of this study is twofold: to review the current literature on the prevalence and risk of tooth wear in PD patients; and to propose hypotheses for future research on this topic.

Material and methods

To address the first aim of the study, a PubMed search using Medical Subject Headings (MeSH) terms "Parkinson's disease" and "tooth wear" was conducted on January 2, 2024. In addition, free terms such as "tooth wear" and "dental wear" were included in the search strategy. Only original articles were analyzed, with no restrictions on language or publication year. Furthermore, the authors conducted a review of the references cited in all included articles.

Results

A total of 8 articles were identified, with only 4 articles describing aspects of tooth wear in PD patients. Magee described a 53-year-old woman with PD who developed tooth wear due to "pursing mouth movements" as a side effect of high levels of levodopa (4.5 g).[11] However, after reducing the dosage, grinding of the teeth was still present. After 10 months, further damage to the teeth was described as "not acceptable". However, the patient was unwilling to end the therapy due to the alleviation of the PD-related symptoms. Splint therapy was then recommended, and after 7 months, no further progression of tooth wear was observed.[11] Furthermore, a questionnaire-based pilot study found a significant association between self-reported tooth wear and the presence of PD.[12] However, the authors discussed the low reliability of self-reported data regarding tooth wear,[12] suggesting that this finding should be interpreted with caution. In a secondary analysis of the questionnaire data from the study by Verhoeff et al.,[12] the authors found that self-reported tooth wear was associated with more sleep and awake bruxism in PD patients.[13] Finally, in another questionnaire-based study with a case-control design, self-reported tooth wear was associated with worse OHRQoL in PD patients compared to matched controls.[6] In summary, the available data on tooth wear in PD patients is limited to self-reporting. Furthermore, the same sample was used in 2 of the 4 included articles. There is a dearth of data on the prevalence of tooth wear in PD patients, and only data on associated factors such as medication, bruxism and OHRQoL is present.

Discussion

The aim of this article was to review the literature on the prevalence and risk of tooth wear in PD patients and to propose hypotheses for future research on this topic. The available studies suggest that tooth wear-related issues are more prevalent in PD patients compared to controls. However, these studies primarily rely on self-reporting. Additionally, only medication usage, bruxism and OHRQoL have been identified as associated factors for tooth wear in PD patients.

Tooth wear is a complex phenomenon with several key aspects that require investigation. These include assessment, epidemiology, etiology, consequences, and management.

A review of the literature revealed that only a few of these aspects have been explored in relation to PD. Given the scarcity of literature on the prevalence of tooth wear in PD patients, this issue should be investigated in representative patient samples and compared with healthy controls using reliable assessment tools.[4] Importantly, risk factors for tooth wear in PD, which are important across all 5 key aspects, have been largely understudied and represent significant gaps in the literature. Addressing these gaps requires well-designed studies in representative PD populations. Drawing insights from established risk factors for tooth wear in the healthy population[14–18] and examining these factors in relation to the various characteristics of PD may provide a further understanding of the risk of tooth wear in PD patients.

Therefore, the following sections will explore the medical, social and miscellaneous risk factors for tooth wear identified in healthy individuals, considering their potential implications for PD. This exercise is intended to propose hypotheses for future research in this field.

Medical risk factors

Slater et al.[17] identified several medical risk factors for tooth wear that are present in the general population. These factors include genetics, orofacial pain, psychology, salivary flow, sleep disorders, medication usage, and stomach acid regurgitation (Table 1). However, no studies have been published regarding the genetics that are hypothesized to be involved in the higher risk development of tooth wear in PD patients. Pain is a common non-motor symptom in PD patients, with a reported prevalence ranging from 68% to 85%.[22] Yet, the presence of pain in the orofacial area, such as temporomandibular disorder (TMD) pain, and its proposed etiology (i.e., bruxism) have not been examined broadly. Nevertheless, a recent scoping review found that both TMD pain and bruxism were more prevalent in PD patients than in healthy controls, with prevalence ranging from 0% to 33% and from 2% to 57%, respectively.[23,24] This suggests that tooth wear due to bruxism may be more prevalent in PD patients. In addition to sleep bruxism, other sleep disorders are associated with PD. For example, both central and obstructive sleep apnea have been mentioned, with prevalence rates of 0–49% and 42–60%, respectively. However, these conditions have not been studied in depth.[25–30] Also, a correlation has been found between sleep parameters, such as the mean oxygen saturation and the percentage of rapid eye movement (REM) sleep, and the severity of PD.[28] However, the proposed mechanism remains unclear. The location of the degeneration in the brain is a potential contributing factor.[31] When sleep disorders are more prevalent, leading to increased arousals and episodes of bruxism, the risk of tooth wear is potentially increased.

Table 1. Risk factors for the presence and/or severity of tooth wear in healthy individuals, along with the hypotheses of the underlying mechanisms

Risk factors		Underlying mechanism
Medical factors	genetics	Genetic variations in, for example, amelogenin, which is involved in the formation of enamel, may be associated with the presence and/or severity of tooth wear.[15,19]
	pain	Pain is an indirect marker for tooth wear. The presence of orofacial pain is indicative of the potential for bruxism, which is associated with an increased risk of tooth wear.[15,17]
	sleep disorders	Nowadays, bruxism is considered a potential protective factor in the context of sleep apnea, with an increased likelihood of tooth wear.[17] Furthermore, RBD is associated with bruxism.[20]
	psychology	Individuals with psychological issues, such as ADHD, anxiety, or stress, are more prone to bruxism, with a higher likelihood of developing tooth wear.[15,17]
	saliva	Saliva plays an essential role in the protection of dental hard tissues. A reduction in salivary secretion or a deterioration in its quality (e.g., an abnormal buffer capacity), increases the likelihood of tooth wear.[14,15,17]
	medication usage	The use of certain medications has an influence on bruxism activity[21] and reduces salivary flow.[17]
	stomach acid regurgitation	In the presence of reflux, the pH of the oral cavity is lower, which in turn leads to erosive tooth wear. The risk of reflux is also higher in individuals with sleep disorders, such as obstructive sleep apnea, or due to dietary intake. However, the dietary intake alone can be responsible for influencing the pH of the oral cavity.[14,15,17,18]
Social factors	stimulant usage (alcohol, caffeine, drugs, smoking)	The use of stimulants has been associated with the aggravation of bruxism and a reduction in the pH of the oral cavity, which in turn increases the risk of tooth wear.[17,18]
	erosive diet	Please refer to the section on stomach acid regurgitation.
	sports	Physical activity may contribute to dry mouth. Additionally, there is a hypothesis that some types of athletes, such as those engaged in strength training, may be prone to clenching.[27]
Miscellaneous factors	ageing	Tooth wear is a progressive phenomenon, the prevalence of which increases with age.[17,18]
	occlusion	Occlusal force and premature contacts may influence the presence and/or severity of tooth wear.[16–18]
	oral hygiene	The type of toothbrush and the frequency of oral hygiene practices may influence the presence and/or severity of tooth wear.[14]

RBD – rapid eye movement sleep behavior disorder; ADHD – attention deficit hyperactivity disorder.

Furthermore, REM sleep behavior disorder (RBD), a parasomnia of PD, is present in 3–60% of PD patients.[32] In patients with RBD, sleep bruxism was found in 25% of the cases,[20,23] which is considerably higher than the prevalence of sleep bruxism observed in the general population. A high prevalence of sleep bruxism may be associated with an increased risk for tooth wear.

Patients with PD are more prone to psychological disorders, such as depression and anxiety, with a prevalence of 38% and 31%, respectively.[33,34] In the general population, psychological disorders are primarily regarded as risk factors for tooth wear due to their association with bruxism. It is therefore possible that the risk of tooth wear is also increased in PD patients.

In addition, salivary problems are more pronounced in PD patients than in their healthy peers. A systematic literature review showed that an objectively lower salivary flow was found in PD patients than in healthy controls.[35] This could be due to the medication used by PD patients. Oonk et al. reported that the number of drugs taken by PD patients is, on average, 7.4 ±2.5 pills per day, with a median of 5 times of drug intake per day.[36] Verhoeff et al. demonstrated that dopaminergic medication, often used to treat PD patients, is probably associated with bruxism and TMD pain.[24] Therefore, in addition to salivary problems, the risk factors of bruxism and TMD pain may be involved in the presence of tooth wear in PD patients due to medication usage.

Gastroesophageal reflux (GERD) was found to be associated with PD (odds ratio (OR): 1.29–4.05).[37,38] In addition, Maeda et al. showed that GERD is present in 26.5% of PD patients.[37] A 65% increase in the incidence of gastrointestinal disorders, including dysphagia, constipation and GERD, was observed in patients with PD within the first 4 years following diagnosis.[39] Thus, as in healthy individuals, reflux is a potential, and perhaps even more significant, risk factor for tooth wear in PD patients.

As previously stated, several medical factors may increase the risk of tooth wear in PD patients, including sleep-related problems. However, it is important to note that awake bruxism is also prevalent (46%) in PD patients.[14] In addition, PD is a movement disorder, which could also be accompanied by phenomena such as dyskinesias (i.e., an involuntary, recurrent, or intermittent movement disorder characterized by fragmentary, jerky, dystonic, or chorea-like movements).[40,41] Therefore, it is hypothesized that more tooth-to-tooth contact could be present in PD patients than in healthy controls, at least in part due to increased craniofacial muscle tone, which may result in the loss of dental hard tissues.

Social risk factors

The social risk factors for tooth wear, and the hypotheses on how these factors may contribute to tooth wear are described in Table 1. Upon examination of the literature, it was found that lifestyle factors such as alcohol consumption are negatively associated with the risk of developing PD.[42,43] Additionally, there may be a negative association between caffeine intake[44] and smoking,[43] and the risk of developing PD. In the healthy population, these lifestyle factors are described as risk factors for tooth wear, as they induce bruxism activity. However, considering the negative association between alcohol and caffeine consumption and the risk of developing PD, it is possible that fewer PD patients use alcohol or caffeine, although this is not necessarily the case. Consequently, the risk of increased tooth wear concerning these factors is likely the same in PD patients as in healthy controls. However, the exact prevalence of alcohol, caffeine and tobacco use among PD patients is not described. Thus, caution is recommended in interpreting the results.

No information regarding the dietary preferences in PD has been documented. However, the intake of particular types of nutrients, such as vitamin C, is known to exert beneficial effects. However, they can also have a counteracting effect (e.g., high-protein and ferrous sulfate supplements) with dopaminergic medication,[45] and thus should be stimulated or discouraged by physicians. Tooth wear is frequently associated with dietary intake, particularly with the consumption of acidic nutrients. However, no research is available on the dietary preferences of PD patients. Nevertheless, we can hypothesize that some factors are important. For example, one of the non-motor symptoms of PD is loss of smell.[7] This can result in a diminished sense of taste, which may lead to the use of more pronounced flavors, including acidity, and thus an increased risk of erosive tooth wear. Furthermore, vitamin C has been suggested to be beneficial for the pharmacokinetics and effectiveness of dopaminergic medication.[45] Therefore, physicians may advise PD patients to take vitamin C supplements or consume more fruits that contain high amounts of vitamin C. However, both have high acidity levels, which could potentially increase the risk of erosive tooth wear.

Finally, PD patients are encouraged to engage in regular physical activity, although there is a paucity of data regarding their use of sports drinks or participation in endurance sports. In healthy athletes, both sports drinks and endurance sports have been identified as risk factors for tooth wear. In particular, endurance sports have been identified as a risk factor for tooth wear, due to the increased clenching that occurs during the activity. Although PD patients are encouraged to engage in sports, it is unlikely that their level of sporting activities will differ significantly from that of healthy controls.

Miscellaneous risk factors

In addition to medical and social factors, aging, dental occlusion and oral hygiene measures may contribute to

increased tooth wear (Table 1). Although the prevalence of PD during younger years is also increasing, the incidence of PD is higher in older individuals.[9] Previous studies have demonstrated that older individuals tend to have worse oral health. This phenomenon is also observed in PD patients, resulting in the loss of teeth or the presence of more root remnants.[10] Factors such as reduced self-care in PD patients may contribute to the etiology of poor oral health. However, a literature review on oral health revealed that oral hygiene frequencies in PD patients were not found to be significantly different from those of healthy controls.[10] It can be hypothesized that because PD patients are informed about their higher risks of poor oral health, they may improve their self-care practices. Furthermore, due to the motor difficulties associated with PD, uncontrolled movements can occur, resulting in abrasion (i.e., tooth wear as a consequence of mechanical causes, other than tooth-to-tooth contact). However, no data is available on oral hygiene practices in individuals with PD.

Conclusions

The existing literature on the prevalence and risk factors of tooth wear in patients with PD is limited. However, it is reasonable to assume that factors such as reduced salivary flow, bruxism, medication usage, and reflux commonly co-occur in PD patients, thereby increasing the risk of tooth wear. Consequently, oral healthcare providers should focus on the risk of more severe tooth wear in PD patients compared to healthy controls, which may further compromise their OHRQoL. However, it remains unclear whether tooth wear is perceived as a problem by PD patients themselves or by their oral healthcare providers.

Based on the findings of this study, the following hypotheses for future studies could be formulated: 1) tooth wear, particularly in severe cases, is more prevalent in PD patients than in healthy controls; 2) risk factors for tooth wear observed in healthy individuals are more prevalent among PD patients; and 3) multiple risk factors for tooth wear co-exist in people with PD, potentially influencing the prevalence and progression of tooth wear in this population.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

This manuscript is a translated and adapted version of the Dutch article entitled “Gebitsslijtage bij de ziekte van Parkinson: Prevalentie en risicofactoren” by Verhoeff MC et al., published in ACTA Quality Practice, 2024, with permission from ACTA Dental Education (Amsterdam, the Netherlands).

ORCID iDs

Merel Charlotte Verhoeff https://orcid.org/0000-0001-7861-5935
Peter Wetselaar https://orcid.org/0000-0002-9443-1260
Frank Lobbezoo https://orcid.org/0000-0001-9877-7640

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Review

Efficiency of cannabis and cannabidiol in managing chronic pain syndromes: A comprehensive narrative review

Marta Bort[1,B–E], Cyprian Olchowy[2,B–D], Anna Olchowy[1,B–D], Izabela Nawrot-Hadzik[3,D], Joanna Smardz[1,D,E], Mieszko Wieckiewicz[1,A,E,F]

[1] Department of Experimental Dentistry, Wroclaw Medical University, Poland
[2] Collegium Medicum, Jan Dlugosz University in Czestochowa, Poland
[3] Department of Pharmaceutical Biology and Biotechnology, Faculty of Pharmacy, Wroclaw Medical University, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):765–782

Address for correspondence
Marta Bort
E-mail: bort.marta@gmail.com

Funding sources
This review was funded by Wroclaw Medical University (grant No. SUBK.B160.24.038 – M. Bort), according to records in the Simple system.

Conflict of interest
None declared

Acknowledgements
None declared

Received on July 16, 2024
Reviewed on August 28, 2024
Accepted on September 6, 2024

Published online on October 31, 2024

Abstract

Chronic pain affects up to 40% of adults, contributing to high medical expenses, the loss of productivity, reduced quality of life (QoL), and disability. Chronic pain requires detailed diagnostic assessment, treatment and rehabilitation, yet approx. 80% of patients report inadequate pain management. As new treatment options are needed, we aimed to explore the effectiveness of medical cannabis-based products in managing chronic pain, with a particular focus on treatment patterns.

We searched the PubMed, Scopus and Web of Science databases using keywords related to cannabinoids and chronic pain syndromes. In total, 3,954 articles were identified, and 74 studies involving 12,562 patients were included. The effectiveness of cannabis-based products varied across studies. Cannabinoids were most effective in treating chronic secondary headache and orofacial pain, chronic secondary musculoskeletal pain, chronic secondary visceral pain, and chronic neuropathic pain. Properly qualifying patients is the first crucial step in managing chronic pain, considering pain characteristics, comorbidities and other treatment options. Treatment should start with low doses of cannabinoids, which are then increased to achieve the desired therapeutic effect while minimizing adverse effects.

This narrative review revealed significant gaps in the evidence regarding precise treatment patterns, particularly for the long-term maintenance treatment needed by patients with chronic pain. Medical cannabis can be considered an option for carefully selected patients with chronic pain syndromes when other treatment options fail to achieve an adequate response, and when the potential benefits outweigh the risks. However, there is still a need for well-designed clinical research to establish the long-term efficacy and safety of cannabinoids.

Keywords: cannabis, cannabinoids, THC, cannabidiol, chronic pain syndrome

Cite as
Bort M, Olchowy C, Olchowy A, Nawrot-Hadzik I, Smardz J, Wieckiewicz M. Efficiency of cannabis and cannabidiol in managing chronic pain syndromes: A comprehensive narrative review. *Dent Med Probl.* 2024;61(5):765–782. doi:10.17219/dmp/193020

DOI
10.17219/dmp/193020

Introduction

The current definition of pain, describing it as "an unpleasant sensory and emotional experience associated with, or resembling that associated with actual or potential tissue damage", was proposed by the Task Force of the International Association for the Study of Pain (IASP) and published in 2020.[1] Pain is recognized as a subjective sensation. However, although it is often connected to a pathological process, it can occur without any tissue damage or clear physiological cause. Furthermore, patients with similar conditions may perceive pain differently. Pain intensity is assessed using patient-reported outcome measures, either as a stand-alone experience or in association with an underlying condition. Pain is categorized into acute and chronic types. Acute pain arises suddenly and typically resolves quickly, whereas chronic pain persists for more than 3 months and often recurs.[2] Chronic pain that lasts or recurs for over 3 months can become the main clinical concern for some individuals, necessitating specific diagnostic evaluation, therapy and rehabilitation. Such a condition is associated with significant distress, contributing to reduced quality of life (QoL), impaired daily functioning and lower productivity at work.[3] It is estimated that in the USA, chronic pain affects 11–40% of adults, contributing to an estimated annual cost of $560 billion in direct medical expenses, the lost productivity and disability support programs.[4] The understanding of pain is expanding due to the categorization based on its origin, such as nociceptive (resulting from a tissue injury), neuropathic (stemming from a nerve injury) or nociplastic (arising from the sensitized nervous system). Differentiating between chronic primary and chronic secondary pain syndromes enables more personalized antipain treatment for patients.[5,6] Guidelines commonly advocate a personalized, multimodal, interdisciplinary treatment strategy encompassing pharmacotherapy, psychotherapy, integrative therapies, and invasive procedures.[5,7] Yet, the percentage of patients not responding to treatment or those who benefit from the proposed strategies only for a limited period is high.[8–10] Nearly 80% of patients report inadequate pain management.[11]

The high burden of chronic pain and the lack of universal treatment prompt researchers to seek new treatment modalities. One of these are cannabis-based medicines. They embrace primarily cannabinoids, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), which interact with the endocannabinoid system (ECS) of the body. This reaction may help reduce pain and inflammation, offering relief to some chronic pain patients. It is also worth mentioning that there are many ways of administering cannabis, like inhalation, oral ingestion and sublingual application, which can be individually selected for particular patients. Recent systematic reviews have analyzed various aspects of cannabis-based medicines, including their efficacy, real-world effectiveness, comparison with other analgesics, and potential for reducing the use of other analgesics. These reviews have led to diverse conclusions.[12–16]

There is a lack of comprehensive analyses of studies specifically assessing the efficacy of cannabis in chronic primary and secondary pain syndromes. Hence, this narrative review aimed to explore the effectiveness of medical cannabis in managing chronic pain, with a particular focus on treatment patterns.

Methods

The search was conducted on April 28, 2024, using the PubMed, Scopus and Web of Science databases. Keywords and synonyms for cannabinoids were considered, including "*Cannabis sativa*", "cannabinoid", "cannabidiol", "CBD", "nabiximols", "marijuana", and "hemp". Regarding chronic pain syndromes, the classification of IASP was used.[6] Referring to pain, the keywords was "chronic pain" and all its types according to the IASP classification, i.e., "chronic primary pain", "chronic cancer-related pain", "chronic postsurgical or post-traumatic pain", "chronic secondary musculoskeletal pain", "chronic secondary visceral pain", "chronic neuropathic pain", and "chronic secondary headache or orofacial pain". Primary original articles reporting results on the efficacy of cannabis and cannabidiol in patients with chronic pain syndromes were considered. The selection of these articles was limited to studies on adult patients. For studies on treatment patterns, additional sources included treatment guidelines and consensus papers. The selection of studies on the mechanism of action aimed to include articles that best explained the pharmacokinetics and mechanism of action of cannabis and cannabidiol, including review papers and animal studies. Additionally, the bibliographies of review papers were screened for the papers potentially omitted in the search. Case reports were excluded due to the low quality of evidence (in connection with evidence-based medicine (EBM)).[17] All the included articles were in the English language. Studies only investigating illegal sources of hemp were not selected for this review. The collection and/or assembly of data, but also data analysis and interpretation were done by 3 authors (M.B., C.O. and A.O.). Information about the study selection and the characteristics of the included studies (including pain syndromes) are presented on Fig. 1 and 2.

Results

Description of the included studies

In total, 3,954 articles were identified, of which 74 were included for qualitative analysis. These studies

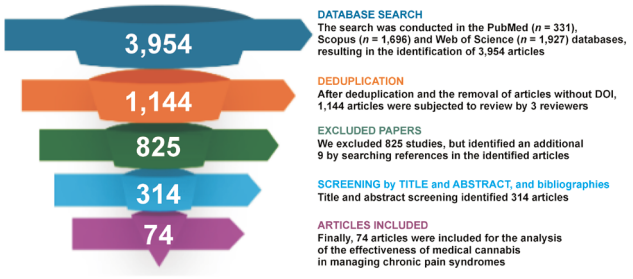

Fig. 1. Flowchart of the study selection process

included 12,562 patients with different chronic pain syndromes. Studies that were not focused on chronic pain syndromes were excluded. Additionally, studies involving pediatric populations, animal studies, laboratory studies, and experimental research were excluded. Regarding the study design, review papers, letters to the editors, book chapters, guidelines, conference proceedings, abstracts, and interviews were not included. Finally, papers in which cannabis and cannabidiol were used only as part of a multi-ingredient preparation were also excluded. The flowchart of the study selection process is shown in Fig. 1.

First, the studies were divided by chronic pain syndrome. Many of the studies included a mixed patient sample, followed by those focusing on chronic secondary musculoskeletal pain and chronic neuropathic pain. However, when considering the number of patients in each chronic pain syndrome, over half of the patients were in the mixed population studies. The distribution of studies and of patients across the included studies is illustrated in Fig. 2A and 2B, respectively.

To assess the effectiveness of medical cannabis in pain reduction, the studies were categorized into 3 groups: those showing the lack of significant improvement in pain indices (⊗); those reporting significant improvement (☑); and those with mixed results leading to inconclusive efficacy conclusions (**?**). Most studies reported significant improvement, followed by those reporting partial improvement. Fewer studies reported negative results. When examining the reported improvement, it is evident that medical cannabis is most effective in managing chronic secondary headache and orofacial pain, chronic secondary visceral pain, chronic secondary musculoskeletal pain, and chronic neuropathic pain. The distribution of studies by their effectiveness is shown in Fig. 3.

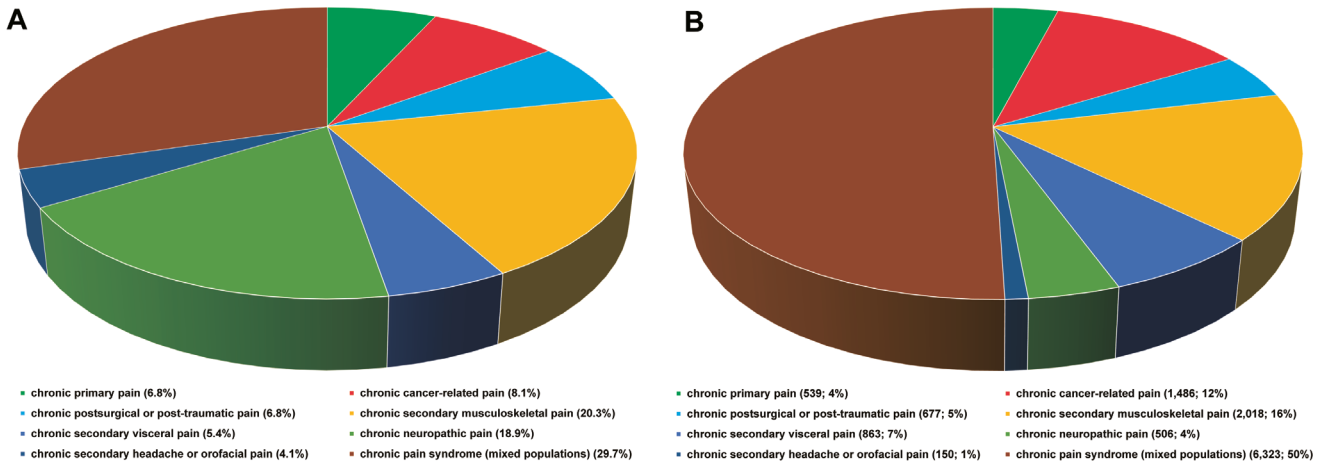

Fig. 2. Distribution of studies (A) and of patients across the included studies (B)

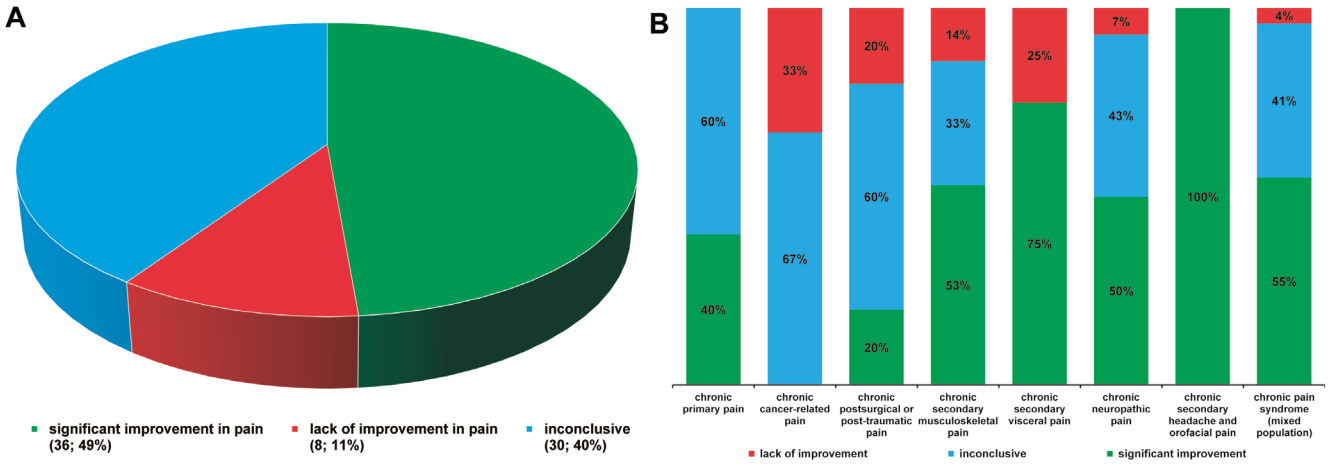

Fig. 3. Distribution of studies by their effectiveness – generally (A) and with regard to the type of chronic pain (B)

Mechanism of action of cannabinoids

Medical cannabis refers to the use of the cannabis plant or its components, such as cannabinoids, like THC and CBD, which interact with the ECS of the body, for medicinal purposes. It is prescribed by healthcare professionals to treat a variety of symptoms and conditions.[18] The terms "medical cannabis" and "medical marijuana"(MM) are often used interchangeably, but they technically refer to different substances they contain and their form. "Cannabis" is the scientific name for a plant species that includes both marijuana and hemp. "Marijuana" specifically refers to strains of cannabis that contain high levels of the psychoactive compound delta-9-tetrahydrocannabinol (THC), which is responsible for the intoxicating effects of the plant. "Hemp", on the other hand, is a strain of cannabis that contains very low levels of THC, and is mostly used for industrial and medical purposes.[19]

Canabis sativa, known for its medicinal properties, contains over 60 unique cannabinoids, each with distinct health benefits. These cannabinoids interact with the ECS of the human body. The most notable cannabinoids are THC, responsible for the psychoactive effects of cannabis, and CBD, recognized for its therapeutic potential and lack of psychoactivity.[20] The mechanism of action of CBD involves interaction with various receptors and signaling pathways in the body, as it interacts with the ECS through multiple pathways.[21]

Unlike THC, CBD does not directly bind to cannabinoid receptors CB1 and CB2, but can inhibit enzymes responsible for breaking down endocannabinoids, leading to increased endocannabinoid levels in the body.[22] Cannabidiol has a low affinity for the orthosteric binding sites of CB1 and CB2 receptors, and exhibits allosteric activity on both receptors. CB1 receptors, primarily found in the central nervous system (CNS), including regions responsible for pain perception, are affected by CBD. Additionally, the antagonistic effects of CBD on CB2 receptors contribute to the anti-inflammatory response by suppressing mast cell degranulation and neutrophil propagation near pain centers.[21] Furthermore, CBD activates transient receptor potential vanilloid type 1 (TRPV1) receptors involved in pain perception, influencing pain sensation and inflammation. Finally, CBD can modulate the levels of neurotransmitters, like serotonin (via serotonin 5-HT1A receptor) and anandamide (via the activation of CB1, CB2 and TRPV1 receptors), indirectly impacting the regulatory functions of ECS.[22] Cannabidiol may also target G-protein-coupled receptor 2 (GPR2), expressed in the brain and spinal cord, which is involved in pain reception.[21] Another pathway explored in experimental research involves the upregulation of matrix metalloproteases (MMP) in spinal cord injuries. Research shows that the inhibition of MMP through TRPV1 and cannabinoid receptors may reduce chronic neuropathic pain.[23]

Efficacy of cannabis in pain syndromes

Chronic primary pain

The features of chronic primary pain include emotional distress caused by pain, impaired daily life activities and reduced social participation.[24] This type of pain was identified in 5 studies: 2 included patients with migraines,[25,26] 2 included patients with fibromyalgia[27,28] and 1 included patients with pain originating in different anatomical regions.[29] In total, the studies included 539 patients. Three studies reported significant pain reduction after treatment with medical cannabis,[26–28] while 2 studies reported high percentages of responders to treatment – 61%[25] and 82%.[29] Only 2 studies utilized a unified treatment protocol. The details of the studies reporting results for chronic primary pain are listed in Table 1.

Chronic cancer-related pain

Patients with chronic cancer-related pain experience this type of pain due to either their active tumor (including metastases) or the oncology treatment they undergo to manage cancer, which may involve surgery, chemotherapy and radiotherapy.[30] We identified 6 studies that involved patients with cancer-related pain.[31–36] These studies included a total of 1,486 patients. None of the studies reported significant improvement in pain across all the conducted comparisons. Two studies revealed that MM was not effective for chronic cancer-related pain.[32,33] All studies, except one, utilized standardized dosing in the treatment schedule. The studies reporting results for chronic cancer-related pain are listed in Table 2.

Chronic postsurgical or post-traumatic pain

Pain that develops or intensifies after a surgical procedure or a tissue injury, such as trauma or a burn, is categorized as chronic postsurgical or post-traumatic pain. This type of pain is characterized by several features – it begins or worsens after surgery, or trauma persists or recurs for more than 3 months, is localized in the affected area, and cannot be attributed to other conditions, including infection, cancer, or the pre-existing pain conditions.[37] The use of MM for pain was investigated in 5 studies.[38–42] These studies included a total of 677 patients. Of the 5 studies included in this category, only one reported significant improvement in response to treatment with CBD.[42] The studies on chronic postsurgical or post-traumatic pain are shown in Table 3.

Chronic secondary musculoskeletal pain

Chronic pain originating in joints, bones, tendons, muscles, the vertebral column, or soft tissue, either spontaneously or due to movement, is classified as chronic secondary

Table 1. Studies reporting results for chronic primary pain

Study	Route and form of administration	Study design	Population	Medication	Effectiveness
Aviram et al.[25] 2020 **?**	NA	cross-sectional study	145 treated patients with comorbid migraine (56 non-responders, 89 responders)	medical cannabis, not standardized	89 (61%) responded to treatment; responders were more likely to consume high doses (7.9–109.5 mg/month) of phytocannabinoid ms_373_15c (n = 27; 60%) and low doses (0–9.9 mg/month) of phytocannabinoid ms_331_18d (n = 28; 62%) as compared to non-responders ($p < 0.05$ and $p < 0.01$, respectively)
Baraldi et al.[26] 2022 **?**	oral route; bedrocan – flos form, bediol – granular form, FM2 – powder form	retrospective study 3 and 6 months	32 patients with chronic migraine	bedrocan, bediol, FM2	after 3 and 6 months, no reduction in the number of migraine days ($p = 0.1182$), but reduced pain intensity ($p = 0.0004$) and acute medication consumption ($p = 0.0006$)
Chaves et al.[27] 2020 ☑	oral route; cannabis oil	double-blind RCT 10 days	17 women with fibromyalgia	THC-rich cannabis oil (24.44 mg/mL of THC and 0.51 mg/mL of CBD	the FIQ pain score improved significantly: cannabis vs. control post-intervention (3.75 vs. 7.67; $p = 0.006$)
Habib and Artul[28] 2018 ☑	NA	retrospective study	26 patients with fibromyalgia	medical cannabis, not standardized	the level of pain before and after treatment (9.21 vs. 3.35; $p < 0.001$)
Habib et al.[29] 2021 **?**	NA	cross-sectional study	319 patients, mainly with fibromyalgia	THC/CBD (18.38 ±4.96% and 2.62 ±4.87%)	in 260 (82%) fibromyalgia patients, the mean pain reduction was 77% with a monthly dose of 31 g

RCT – randomized clinical trial; THC – tetrahydrocannabinol; CBD – cannabidiol; FIQ – Fibromyalgia Impact Questionnaire; ⊗ – all results non-significant; ☑ – all results significant; **?** – some results significant or no statistical comparison conducted; NA – data not available.

Table 2. Studies reporting results for chronic cancer-related pain

Study	Route and form of administration	Study design	Population	Medication	Effectiveness
Aviram et al.[31] 2020 **?**	sublingual and inhalational routes; medical cannabis oil extract, inflorescence inhalation	multicenter, prospective study 3 and 6 months	108 patients with treatment for metastatic cancer pain, and for chemotherapy-related nausea, vomiting, and/or pain	3 types of medication (THC dominant, CBD dominant, THC/CBD)	the weekly least and worst pain intensity improved not significantly ($p = 0.27$ and $p = 0.10$), significant improvement in the weekly average pain intensity ($p < 0.05$), affective pain intensity ($p < 0.01$), sensory pain intensity ($p < 0.05$), and the PCS score ($p = 0.47$)
Fallon et al.[32] 2017 ⊗	sublingual and buccal routes; aerosol for use in the oral cavity	2 phase 3, double-blind RCTs	399 advanced cancer patients with chronic pain unalleviated by optimized opioid therapy	adjunctive sativex; part A (sativex, 10.1%) and part B (sativex, 27.2%; placebo, 10.7%)	the mean average pain scores increased from 3.2 to 3.7 in the sativex group and from 3.1 to 3.6 in the placebo group, no differences in the worst pain NRS scores between the study groups
Fehniger et al.[33] 2021 ⊗	NA	retrospective study, median: 5.2 months	45 gynecologic cancer patients	MM	36% of patients using MM for pain relief
Johnson et al.[34] 2010 **?**	sublingual and buccal routes; oromucosal spray	multicenter, double-blind RCT 2 weeks	177 patients with moderate to severe cancer-related pain	THC, THC:CBD, placebo	the median changes from baseline for THC, THC:CBD and placebo were −1.00, −1.36 and −0.60, respectively, the adjusted mean treatment difference from placebo was significant for a reduction in pain with the THC:CBD extract (0.67 points, $p = 0.014$), but not the THC extract (0.32 points, $p = 0.245$)
Lichtman et al.[35] 2018 **?**	sublingual and buccal routes; aerosol for use in the oral cavity	phase 3, double-blind RCT 5 weeks	397 advanced cancer patients	sativex	the median percent improvement in the NRS pain score between baseline and the end of treatment in the nabiximols and placebo groups was 10.7% vs. 4.5% ($p = 0.0854$) in the intention-to-treat population (primary variable) and 15.5% vs. 6.3% ($p = 0.0378$) in the per-protocol population, nabiximols were statistically superior to placebo in week 3, as measured with 2 of 3 quality-of-life instruments, and in week 5, as measured with all 3 instruments
Portenoy et al.[36] 2012 **?**	sublingual and buccal routes; aerosol for use in the oral cavity	graded-dose RCT	360 advanced cancer patients	sativex	the 30% responder rate primary analysis was not significant for nabiximols vs. placebo ($p = 0.59$), a secondary continuous responder analysis of the average daily pain from baseline to the end of the study: The proportion of patients reporting analgesia was greater for nabiximols than placebo – overall ($p = 0.035$), and specifically in the low-dose ($p = 0.008$) and medium-dose ($p = 0.039$) groups

MM – medical marijuana; PCS – Pain Catastrophizing Scale; NRS – numeric rating scale; ⊗ – all results non-significant; ☑ – all results significant; **?** – some results significant or no statistical comparison conducted; NA – data not available.

Table 3. Studies reporting results for chronic postsurgical or post-traumatic pain

Study	Route and form of administration	Study design	Population	Medication	Effectiveness
Cardenas and Jensen[38] 2006 ?	NA	postal survey	117 patients with SCI	mixed	MM provided greater pain relief by 6.62 ±2.54 (scores rated from 0 to 10)
Cuñetti et al.[39] 2018 ?	oral route; oral solution	study design not provided 3 weeks	7 patients after kidney transplantation	CBD	2 patients had total pain improvement, 4 had a partial response in the first 15 days and in 1 there was no change
de Vries et al.[40] 2017 ⊗	oral route; tablets	phase 2 RTC 50–52 days	65 patients with chronic abdominal pain after surgery or due to chronic pancreatitis	THC	the VAS mean scores did not differ significantly between the THC and placebo groups ($p = 0.901$), between the start and the end of the study, the VAS mean scores decreased by 1.6 points (40%) in the THC group as compared to 1.9 points (37%) in the placebo group
Greis et al.[41] 2022 ?	NA	prospective, observational study 12 months	468 orthopedic pain patients	medical cannabis	the VAS pain score was significantly reduced at 3, 6 and 12 months (6.7 vs. 5.2 at the first follow-up; $n = 385$, $p < 0.001$), there were no significant differences in the VAS pain scores between follow-ups at 3, 6 and 12 months
Hall et al.[42] 2023 ☑	transdermal route; cream for lower extremities	retrospective study 6 weeks	20 patients with chronic pain resulting from acute lower extremity injuries	topical CBD	there was significant improvement in the self-reported pain levels (intake mean: 3.5 ±0.29, exit mean: 1.7 ±0.23; $p < 0.001$) and pain-related disability ($p < 0.001$)

SCI – spinal cord injury; VAS – visual analog scale; ⊗ – all results non-significant; ☑ – all results significant; ? – some results significant or no statistical comparison conducted; NA – data not available.

musculoskeletal pain.[43] This type of pain can develop due to a musculoskeletal disease with inflammation caused by infection, autoimmunity, autoinflammation, or metabolic disorders, a musculoskeletal disease with structural or biomechanical factors, or a neurological disease that alters the biomechanical function.[43] The use of MM for chronic secondary musculoskeletal pain was investigated in 15 studies.[44–58] These studies included a total of 2,018 patients. More than half of the studies ($n = 8$) reported significant improvement in pain.[46–49,53,55–57] The studies on chronic secondary musculoskeletal pain are shown in Table 4.

Chronic secondary visceral pain

Patients classified with chronic secondary visceral pain exhibited specific characteristics: the pain arose from particular internal organs; their medical history indicated dysfunction or a disease in one or more internal organs; and the pain could not be explained by any other diagnosis of chronic pain.[59] Four studies included patients who met the criteria for suffering from chronic secondary visceral pain.[60–63] These studies included a total of 863 patients. Of the 4 studies included in this category, only 2 reported significant improvement in response to treatment with CBD,[61,63] whereas 1 study reported preliminary evidence with regard to the in efficacy of treatment. The last one showed no significant reduction of pain.[60] The studies reporting results for secondary visceral pain are shown in Table 5.

Chronic neuropathic pain

This category comprised studies involving patients who experienced chronic pain resulting from conditions that damage the somatosensory nervous system. Chronic neuropathic pain is characterized by a history of neurological lesions or disease, the consistent neuroanatomical distribution of pain sensation, and the presence of sensory signs in the affected area.[64] This pain may be caused by, among other things, diabetic neuropathy, a neurodegenerative, vascular or autoimmune condition, a tumor, trauma, infection, exposure to toxins, or a hereditary disease.[64] In our review, we identified 14 studies investigating chronic neuropathic pain in a total of 506 patients.[65–78] Seven studies reported satisfactory results,[65–71] 1 study showed unfavorable results,[72] and the remaining 6 studies reported inconsistent results after treatment with THC and CBD.[73–78] Table 6 presents the list of studies on chronic neuropathic pain.

Chronic secondary headache and orofacial pain

Chronic secondary headache and orofacial pain encompass all headache and orofacial pain conditions with underlying causes occurring on at least half of the days for a minimum of 3 months, with each episode lasting at least 2 h.[79] This type of headache may be diagnosed when another disorder known to cause headache or orofacial pain has been identified, supported by evidence demonstrating causation. This means that headache or orofacial pain correlates with the progression or regression of the presumed causative disorder.[79] Three studies were included in this group, with a total of 150 patients.[80–82] In 1 study, significant improvement in pain and better results as compared to ibuprofen were reported.[80] The remaining 2 studies reported significant improvement in pain after the topical use of CBD in patients with temporomandibular disorders (TMD).[81,82] Table 7 shows the

Table 4. Studies reporting results for chronic secondary musculoskeletal pain

Study	Route and form of administration	Study design	Population	Medication	Effectiveness
Bakewell et al.[44] 2022 ?	oral route; CBD gel caps	observational study, 6 visits	48 patients with LBP caused by lumbar spinal stenosis	CBD	the usual pain levels and the worst pain levels demonstrated significant improvement ($p < 0.001$ and $p < 0.0015$, respectively), while the pain right now and the best pain level did not improve significantly ($p > 0.05$)
Campbell et al.[45] 2023 ⊗	oral route; oral capsules	double-blind RCT 4 weeks	37 patients with knee osteoarthritis	hydromorphone, dronabinol, placebo	no significant analgesic effects were observed for clinical pain severity or physical functioning across all drug conditions
Corey-Bloom et al.[46] 2012 ☑	inhalational route; smoked cannabis	RCT 2 weeks	37 patients with multiple sclerosis and pain due to spasticity	THC, placebo	the VAS pain score improved after THC (16.61 vs. 8.34), the mean difference as compared to placebo was significant (8.27 vs. 2.90; $p = 0.008$)
Fari et al.[47] 2023 ☑	oral route; hemp seed oil in soft-gel capsules	double-blind, prospective case–control study 45 days	38 patients with knee osteoarthritis	hemp vs. hemp with caryophyllene, myrcene, ginger extract	the NRS pain score in the hemp group dropped from 7.6 ±1.4 to 5.7 ±1.2 ($p < 0.0001$)
Frane et al.[48] 2022 ☑	NA	cross-sectional study	428 with arthritis and joint pain	CBD	CBD users reported that their average daily pain was much better (37.9%) and a little better (45.1%), patients reported a 44% (2.58-point) reduction in the NRS pain score after CBD use ($p < 0.001$), improvement in pain was related to greater frequency of CBD use and longer treatment ($p < 0.001$)
Glare et al.[49] 2023 ☑	oral route; oil	single-arm, open-label study 35 days	40 patients with chronic back or neck pain	cybis	there was dose-dependent improvement in the NRS pain score ($p < 0.001$), with a clinically significant reduction in pain at 1.0 mL bd and 1.5 mL bd doses (a reduction by 28.8% and 34.1%, respectively; $p < 0.001$)
Greis et al.[50] 2022 ?	sublingual and transdermal routes; sublingual tincture and/or topical cannabinoids on legs/lower back	retrospective database study 9 months	186 patients with chronic back pain	medical cannabis	as compared to baseline, the VAS pain score decreased from 73.1 to 58.1, 53.2 and 51.9 at 3, 6 and 9 months, respectively ($p < 0.01$), pain intensity decreased from 7.5 to 6.0, 5.8 and 5.7, respectively ($p < 0.01$), pain frequency decreased from 7.8 to 6.4, 6.2 and 5.6, respectively ($p < 0.01$), insignificant pain drops included: radiating right leg pain; radiating left leg pain; leg pain intensity; and leg pain frequency
Gustavsen et al.[51] 2021 ?	oral route; cannabis oil	prospective, observational safety study 4 weeks	32 multiple sclerosis patients	THC, CBD, THC+CBD	for THC, pain decreased from a median NRS score of 7 to 4 ($p = 0.01$), for CBD, pain decreased from a median NRS score of 7 to 5 ($p = 0.10$)
Pramhas et al.[52] 2023 ⊗	oral route; capsules	double-blind RTC 8 weeks	83 patients with knee osteoarthritis	CBD	the mean reduction in the WOMAC pain subscale scores was 2.5 (95% *CI*: 1.8–3.3) in the CBD group and 2.4 (95% *CI*: 1.7–3.2) in the placebo group, with no significant difference between the groups ($p = 0.80$), the mean reduction in the weekly VAS pain score was 1.9 (95% *CI*: 1.1–2.7) in the CBD group and 2.4 (95% *CI*: 1.6–3.2) in the placebo group, with a mean group difference of −0.51 (95% *CI*: −1.5–0.5) ($p = 0.30$)
Renslo et al.[53] 2022 ☑	sublingual and transdermal routes; sublingual tincture and/or topical cannabinoids	prospective, cohort study 6 months	40 patients with osteoarthritis	medical cannabis	the VAS pain score decreased significantly from 6.6 at baseline to 5.0 at 3 months ($p < 0.01$) and 5.4 at 6 months ($p < 0.05$)
Robinson et al.[54] 2022 ?	sublingual and inhalational routes; sublingual extract, smoked inflorescence	observational, open-label study 2 × 12 months	24 patients with LBP	THC and CBD	the VAS pain score decreased for all participants overall during the study from 83.3 ±15.4 at baseline to 39.1 ±18.5 at 24 months ($p < 0.001$), during the extract therapy phase, this decrease was not significant and averaged 12.3% (*SE*: 5.8, 95% *CI*: −5.3–29.8); changes in VAS were significant at 12–24 months and 12–18 months, which was attributed to the superiority of the inhalation of cannabis as compared to cannabis extract

Study	Route and form of administration	Study design	Population	Medication	Effectiveness
Rog et al.[55] 2005 ☑	sublingual and buccal routes; oromucosal spray	double-blind RCT 4 weeks	66 patients with central pain in multiple sclerosis	CBM, placebo	CBM was superior to placebo in reducing the mean pain intensity (CBM: mean change: –2.7, 95% *CI*: –3.4 to –2.0; placebo: mean change: –1.4, 95% *CI*: –2.0 to –0.8; $p < 0.005$)
Wissel et al.[56] 2006 ☑	oral route; capsules	placebo-controlled, double-blind crossover study 4 weeks each	13 patients with spasticity-related pain in multiple sclerosis	nabilone, placebo	the score in the 11-point-Box Scale test (a measure of spasticity-related pain) decreased by a median of 2 points with nabilone as compared to placebo treatment ($p < 0.05$), whereas placebo treatment showed no change ($p = 0.8$)
Zajicek et al.[57] 2003 ☑	oral route; cannabis extract	RCT 15 weeks	667 patients with stable multiple sclerosis	cannabis extract, THC, placebo	improvement in pain: cannabis extract (46%); THC (50%); and placebo (30%), no change: cannabis extract (32%); THC (33%); and placebo (41%), deterioration: cannabis extract (22%); THC (17%); and placebo (30%); a significant difference ($p = 0.002$)
Zajicek et al.[58] 2012 ?	oral route; capsules	RCT 12 weeks	279 patients with stable multiple sclerosis	cannabis extract, placebo	responders with regard to body pain at 4 weeks (28.0% vs. 17.2%; $p < 0.005$), at 8 weeks (30.1% vs. 19.4%; $p < 0.003$) and at 12 weeks (28.0% vs. 18.7%; not significant)

LBP – low back pain; CBM – whole-plant cannabis-based medicine; WOMAC – Western Ontario and McMaster Universities Index of Osteoarthritis; *CI* – confidence interval; *SE* – standard error; ⊗ – all results non-significant; ☑ – all results significant; ? – some results significant or no statistical comparison conducted; NA – data not available.

characteristics of studies on chronic secondary headache and orofacial pain.

Chronic pain investigated in mixed patient groups

Overall, 22 studies with 6,323 patients reported results for patients with more than one type of chronic pain syndrome.[7,83–103] This group was summarized separately. Twelve studies reported that medical cannabis relieved pain successfully,[83–94] 1 study reported negative results[95] and the remaining 9 studies reported inconclusive results.[7,96–103] The studies reporting results for chronic pain investigated in mixed patient groups are listed in Table 8.

Cannabis treatment patterns for chronic pain

Overall, 36 studies showed a significant reduction in pain, and were further reviewed to identify the most effective treatment patterns. However, after excluding studies using mixed treatment, those shorter than 4 weeks and those involving fewer than 20 patients, only 17 studies were available.[42,49,53,55,57,68,70,80,84,86–89,91–94] The analysis of treatment approaches identified distinct phases in the treatment pathway for reducing pain in patients with chronic pain syndromes, which is illustrated in Fig. 4.

Table 5. Studies reporting results for chronic secondary visceral pain

Study	Route and form of administration	Study design	Population	Medication	Effectiveness
Abrams et al.[60] 2020 ⊗	inhalational route; inhaled vaporized cannabis	RCT 5 days	23 with SCD with chronic pain	THC and CBD, placebo	the mean difference in pain rating assessment between the cannabis and placebo groups was –5.3 ±8.1 for day 1, –10.9 ±7.0 for day 2, –16.5 ±9.2 for day 3, –8.9 ±6.7 for day 4, and –8.2 ±8.1 for day 5; however, none was significant, the mean difference in pain interference rating was not significant
Armour et al.[61] 2019 ☑	oral route; oil	cross-sectional online survey	484 women with endometriosis	medical cannabis	among the self-management modalities, cannabis was rated to bring the highest self-reported pain relief on an 11-item pain relief scale (0–10) with a score of 7.6 ±2.0
Tripp et al.[62] 2014 ☑	sublingual and buccal routes, inhalational, transdermal and rectal routes; smoked, sublingual spray, a vaporizer, an inhaler, rectal suppositories, skin patches, hashish	cross-sectional online survey	342 men with chronic prostatitis/ chronic pelvic pain syndrome	medical cannabis	the effectiveness of cannabis was rated "somewhat/ very effective" by 57% of patients recruited in the urology clinic and by 63% of patients recruited online
Yacyshyn et al.[63] 2020 ☑	oral route; tablets	phase 2a study 8 weeks	14 patients with chronic abdominal pain associated with Crohn's disease	olorinab (25 mg or 100 mg)	at week 8, the mean change from baseline in AAPS at peak olorinab plasma concentrations was –4.61 ±1.77 in the 25-mg group ($p = 0.0043$) and –4.57 ±2.17 in the 100-mg group ($p = 0.0036$), the change from baseline at week 8 in the mean number of pain-free days per week was 1.60 ±2.61 in the 25-mg group and 2.33 ±3.62 in the 100-mg group

SCD – sickle cell disease; AAPS – average abdominal pain score; ⊗ – all results non-significant; ☑ – all results significant; ? – some results significant or no statistical comparison conducted.

Table 6. Studies reporting results for chronic neuropathic pain

Study	Route and form of administration	Study design	Population	Medication	Effectiveness
Abrams et al.[65] 2007 ☑	inhalational route; smoked, pre-rolled cannabis	RCT 5 days	50 patients with HIV-associated sensory neuropathy	cannabis (3.56% THC)	smoked cannabis reduced daily pain by 34% (*Me*) (*IQR*: −71 to −16) vs. 17% (*IQR*: −29–8) with placebo ($p = 0.03$), a greater than 30% reduction in pain was reported by 52% in the cannabis group and 24% in the placebo group ($p = 0.04$), the first cannabis cigarette reduced chronic pain by a median of 72% vs. 15% with placebo ($p < 0.001$)
Eisenberg et al.[66] 2014 ☑	inhalational route; an inhaler	single-dose, open-label phase 1a study 2 h	8 patients with chronic neuropathic pain	Syqe® inhaler device with THC	a significant 45% reduction in pain intensity was noted 20 min post inhalation ($p = 0.001$), turning back to baseline within 90 min
Ellis et al.[67] 2009 ☑	inhalational route; smoked active cannabis	phase 2, single-group, double-blind, placebo-controlled crossover trial 2 × 5 days	28 patients with HIV-associated neuropathic pain	THC, placebo	pain reduction was significantly greater with cannabis as compared to placebo (median difference in pain reduction: −3.3 DDS points; $p = 0.016$)
Kluwe et al.[68] 2023 ☑	inhalational route; dried flowers	retrospective study 6 weeks	99 patients with neuropathic pain, a high severity of symptoms and exhausted treatment options	dried flowers (<12–22% of THC)	the median of the pain scores decreased from 7.5 to 4.0 ($p < 0.001$), the proportion of patients with severe pain (score >6) decreased from 96% to 16% ($p < 0.001$)
Mondello et al.[69] 2018	oral route; oleic suspension	retrospective study 12 months	11 patients with failed back surgery syndrome refractory pain diagnosed with neuropathic pain	THC/CBD combination	the mean pain score decreased from 8.18 ±1.07 to 4.72 ±0.9 ($p < 0.001$)
Toth et al.[70] 2012 ☑	oral route; capsules	double-blind RTC 4 weeks	26 patients with diabetic peripheral neuropathic pain	adjuvant nabilone, placebo	85% of patients on nabilone experienced ≤30% pain reduction as compared to 38% of patients on placebo ($p < 0.05$), for achieving ≤50% pain reduction, it was 31% vs. 8% ($p > 0.5$), at the end of the study, the NRS pain scores were 3.5 ±1.3 for nabilone and 5.4 ±1.7 for placebo, with a mean difference of 3.0 ±1.2 for nabilone and 1.1 ±1.5 for placebo ($p < 0.01$)
Turcotte et al.[71] 2015 ☑	oral route; capsules	RCT 9 weeks	14 patients with multiple sclerosis-induced neuropathic pain	adjuvant nabilone, placebo	a significant group × time interaction term was reported for both the VAS pain ($p < 0.01$) and VAS impact ($p < 0.01$) score, demonstrating that the adjusted rate of decrease for both outcomes was statistically greater in the nabilone group as compared to the placebo group
Rintala et al.[72] 2010 ⊗	oral route; capsules	randomized, controlled, double-blind, crossover pilot study 2 × 12 days	5 patients with central neuropathic pain after SCI	dronabinol, diphenhydramine	changes in pain from baseline to the end of the maintenance phase did not differ between the 2 medications (dronabinol: 0.20 ±0.837, diphenhydramine: −1.80 ±2.490; $p = 0.102$)
van Amerongen et al.[73] 2018 ?	oral route; tablets	crossover RCT 6 weeks	24 patients with progressive multiple sclerosis	THC, placebo	pain rating was significantly reduced overall during 4 weeks of treatment (2.74 for active treatment vs. 4.25 for placebo; $p = 0.0198$), when pain was measured with a daily diary at home, no significant treatment effect was observed (−0.47; 95% *CI*: −2.66–1.71; $p = 0.6581$)
Wade et al.[74] 2006 ?	sublingual and buccal routes; aerosol for use in the oral cavity	open-label, placebo-controlled study 12 months	137 patients with multiple sclerosis	sativex	pain on the VAS scale at baseline vs. 66 weeks in 47 responders (68.1 ±10.6 vs. 26.4 ±18.7), overall, 42.3% withdrew due to the lack of efficacy
Wallace et al.[75] 2015 ?	inhalational route; inhaled vaporized cannabis	crossover RCT 3 h	16 patients with painful diabetic peripheral neuropathy	placebo, doses of THC – low (1%), medium (4%) and high (7%)	the comparison of spontaneous pain over time showed significant differences in the pain scores between the doses ($p < 0.001$), specific significant comparisons were placebo vs. low ($p = 0.031$), medium ($p = 0.040$) and high ($p < 0.001$) dose, and high dose vs. low and medium doses (both $p < 0.001$), it was effective with medium and high doses for up to 2 h
Ware et al.[76] 2010 ?	inhalational route; inhaled through a pipe	crossover RCT 14 days	21 patients with chronic neuropathic pain	placebo, and 2.5%, 6.0% and 9.4% THC	the daily average pain intensity was significantly lower on 9.4% THC than on placebo (5.4 vs. 6.1; $p = 0.023$), the drop in pain for lower concentrations of THC was not significant

Study	Route and form of administration	Study design	Population	Medication	Effectiveness
Wilsey et al.[77] 2008 ?	inhalational route; smoked, cannabis cigarettes	crossover RCT three 6-hour experimental sessions	38 patients with central and peripheral neuropathic pain	placebo, and 3.5% and 7.0% cannabis	significant analgesia (a 0.0035 reduction in VAS pain intensity/min) was noted for 3.5% and 7.0% cannabis vs. placebo ($p = 0.016$), although a trend for the separation of the active agents from placebo is visible by the time of 120 min, significant separation for a specific time point occurred only after a cumulative dose of 9 puffs at 240 min ($p = 0.02$)
Xu et al.[78] 2020 ?	transdermal route; CBD cream applied to the symptomatic area	crossover RCT 4 weeks	29 patients with peripheral neuropathy	CBD, placebo	significant reductions in intense ($p = 0.009$), sharp ($p < 0.001$) and itchy ($p = 0.001$) sensations, and surface pain sensations ($p = 0.013$), no significant reduction in deep pain was observed ($p = 0.064$)

Me – median; *IQR* – interquartile range; DDS – Descriptor Differential Scale; ⊗ – all results non-significant; ☑ – all results significant; **?** – some results significant or no statistical comparison conducted.

Table 7. Studies reporting results for secondary headache and orofacial pain

Study	Route and form of administration	Study design	Population	Medication	Effectiveness
Pini et al.[80] 2012 ☑	oral route; capsules	crossover RCT, 8 weeks	30 patients with medication overuse headache	ibuprofen, nabilone	nabilone was more effective than ibuprofen in reducing pain intensity ($p < 0.05$), the VAS pain scores: 7.9 ±1.6 (baseline); 5.7 ±1.9 (nabilone); 6.6 ±2.2 (ibuprofen); and 6.2 ±2.4 (follow up)
Walczyńska-Dragon et al.[81] 2024 ☑	buccal route; CBD gel for both masticatory muscles intraorally	RCT 14 days	60 patients with TMD	placebo, and 5% and 10% CBD	pain reduction on the VAS scale was 40.8% ($p < 0.05$) in patients using a 5% CBD formulation and 57.4% ($p < 0.05$) in those using a 10% CBD formulation
Nitecka-Buchta et al.[82] 2019 ☑	transdermal route; topical application of CBD cream on the masseter muscle	RCT 14 days	60 patients TMD	CBD, placebo	pain intensity decreased significantly on the VAS scale by 70.2% in the CBD group and by 9.81% in the placebo group

TMD – temporomandibular disorders; ⊗ – all results non-significant; ☑ – all results significant; **?** – some results significant or no statistical comparison conducted.

The qualification of patients is the first key step for patients with chronic pain. Factors that should be considered include the type of the main diagnosis of pain syndrome, the co-occurrence of other conditions that could improve alongside pain,[55,57,88] and exhausted treatment options.[68] The initiation of treatment should be discussed with the patient and based on shared decision-making. Treatment goals can include not only the reduction of pain, but also the improvement of other symptoms and the reduction of opiate and other analgesic intake.[80,86]

A personalized approach to setting the dose, type and route of administration of cannabinoids is underscored in the included studies. Most studies identified a combination of THC and CBD as the most frequent type of effective treatment for chronic pain syndromes. Researchers recommend starting with low doses of cannabinoids and slowly adjusting the doses to reach the desired therapeutic effect.[86] Dose adjustment is made by patients based on the perceived level of pain. Self-titration did not lead to the use of maximal doses allowed in the trials, but varied across the studies. In an RCT by Rog et al., patients could increase the intake of cannabis-based medicine (CBM) to a maximum dose of THC 130 mg:CBD 120 mg; however, the mean final dose was 25.9 mg of THC and 24 mg of CBD.[55] Increasing doses were also used for patients who were prescribed synthetic CB1 receptor agonists (nabilone). In a study by Toth et al., nabilone was started at a dose of 0.5 mg twice daily for 1 week and increased to a maximum dose of 2.0 mg twice daily.[70]

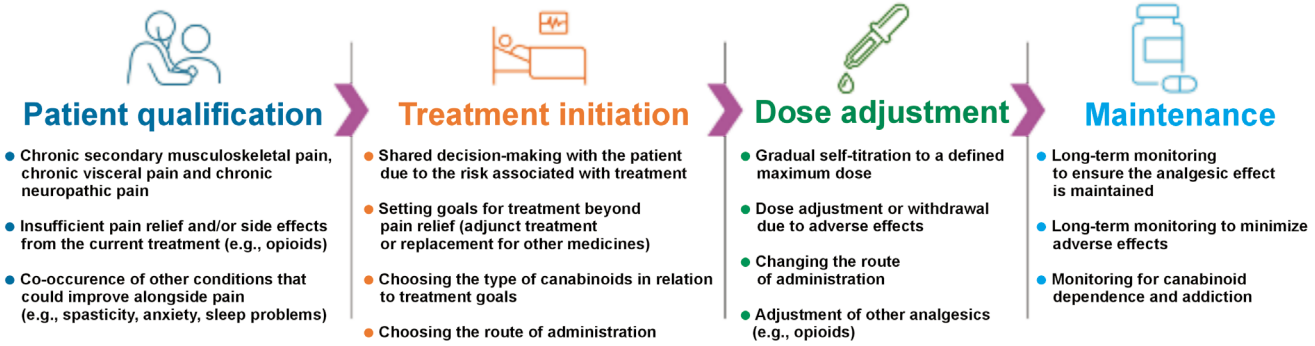

Fig. 4. Distinct phases in the treatment pathway for reducing pain in patients with chronic pain syndromes

Table 8. Studies reporting results for mixed patient groups

Study	Route and form of administration	Study design	Population	Medication	Effectiveness
Abelev et al.[7] 2022 ?	oral route; oil	prospective, observational, open-label study 3 months	71 patients with chronic refractory pain	THC, CBD (LGP Classic 10:10)	a significant decrease in the pain impact score was found, with the mean impact score reduced by 2.3 ±9.4 points ($p = 0.034$), the PROMIS-29 domains of pain score did not improve significantly (6.3 ±2.1 vs. 5.7 ±2.3; $p = 0.053$)
Almog et al.[83] 2020 ☑	inhalational route; an inhaler	RCT 150 min	21 patients with chronic focal or distal symmetric (diabetic) neuropathic pain, and 6 with complex regional pain syndrome	placebo, and THC 0.5 mg and 1 mg Syqe® inhaler	the reduction in the VAS pain score was significantly greater for 1 mg THC as compared to placebo ($p = 0.0015$) and 0.5 mg THC ($p = 0.0058$), the number of patients whose VAS pain score was reduced by ≤30% reached the maximum 120 min post inhalation
Aviram et al.[84] 2021 ☑	sublingual and inhalational routes; inflorescence for smoking/inhaling or oil extract (sublingual use)	prospective study 12 months	551 patients with chronic pain continued the study at 12 months	medical cannabis	the weekly average pain intensity reduced by 20%, from 8 (7–9) to 6 (5–8) (*OR*: −1.97, 95% *CI*: −2.13 to −1.81; $p < 0.001$), the least pain intensity declined by 33%, from 6 (4–8) to 3 (2–6) (*OR*: −1.88, 95% *CI*: −2.08 to −1.67; $p < 0.001$) and the worst pain intensity by 21%, from 9 (8–10) to 8 (6–9) (*OR*: −1.36, 95% *CI*: −1.52 to −1.21; $p < 0.001$)
Balestra et al.[85] 2023 ☑	oral, sublingual and inhalational routes; capsules, aerosol for use in the oral cavity, cannabis flos, cannabinoid flowers	retrospective study	64 patients with chronic pain conditions lasting at least 6 months	medical cannabis	changes before vs. under treatment in the mean pain intensity (6.7 ±1.8 vs. 5.6 ±2.0; $p < 0.001$), pain-associated disability (6.9 ±2.2 vs. 5.8 ±2.4; $p < 0.001$) and pain tolerability (3.3 ±0.7 vs. 2.9 ±0.8; $p < 0.001$)
Crowley et al.[86] 2018 ☑	sublingual and buccal routes; cannabinoids via aerosol	observational study 12 weeks	49 patients with chronic non-cancer pain	Trokie® lozenges	a mean reduction in pain intensity on NRS of 4.9 ±2.0 points was observed (from 7.4 ±1.3 to 2.4 ±1.8; $p < 0.0001$)
Harris et al.[87] 2022 ☑	oral route, sublingual and buccal routes, inhalational route; tablets, a vaporizer, aerosol	database study 6 months	190 patients with chronic pain from the UK Medical Cannabis Registry	CBM	significant improvement was observed within BPI for pain severity and pain interference, in all domains of SF-MPQ-2, the EQ-5D-5L index for pain and discomfort, and VAS measures at all time points ($p < 0.050$)
Horsted et al.[88] 2023 ☑	oral route; oil or capsules	retrospective study *Me*: 126 days	826 patients with chronic refractory pain insufficiently controlled by conventional analgesics or experiencing intolerable adverse events from those	THC, CBD, THC:CBD	the reduction on NRS was significantly different at both follow-up consultations as compared to baseline ($p < 0.0001$), clinically relevant pain reduction (NRS ≥ 30%) was reported by 17% at follow-up 1 and by 10% of patients at follow-up 2 in intention-to-treat analysis, whereas the figures were 32% and 45%, respectively, in per-protocol analysis
Kawka et al.[89] 2021 ☑	oral route; oil	database study 6 months	110 patients from the UK Medical Cannabis Registry	Adven® oil preparation	significant improvement was demonstrated in the EQ-5D-5L pain and discomfort subscale score, the VAS pain score, and BPI at 1, 3 and 6 months ($p < 0.05$)
Narang et al.[90] 2008 ☑	oral route; capsules	crossover RCT: phase 1 – 8 h phase 2 – 1 week	30 patients taking opioids for chronic non-cancer pain	placebo, and adjuvant 10 mg and 20 mg dronabinol	phase 1: total pain relief for placebo (31.1), for 10 mg dronabinol (39.7; $p < 0.05$) and 20 mg dronabinol (41.7; $p < 0.001$), the pain intensity difference was −6.4 for placebo, −17.4 for 10 mg dronabinol ($p < 0.001$) and −19.7 for 20 mg dronabinol ($p < 0.001$) phase 2: a significant decrease in the average pain scores as compared to baseline ($p < 0.001$), there was also a significant change from baseline in the measures of pain and pain relief ($p < 0.01$), in BPI pain interference, a decrease by 1.48 points was found ($p < 0.05$)
Poli et al.[91] 2018 ☑	inhalational route; cannabis flos	prospective study 12 months	338 patients with different chronic pain conditions	cannabis flos, 19% decoction	the VAS pain intensity score dropped significantly between baseline and 12 months (*Me*: 9 vs. 5; $p < 0.001$), the median pain disability score at baseline was 6.28 and decreased to 5.93 ($p < 0.01$), the results improved over the first 3 months, and then remained stable
Pud et al.[92] 2024 ☑	oral route; cannabis oil	prospective study 6 months	218 patients with chronic pain	THC:CBD	52 (24%) patients reported a ≤30% reduction from baseline in their weekly average pain at least at 1 follow-up time point, significant differences in comparisons between baseline and 12 months: weekly pain (7.9 ±1.7 vs. 6.6 ±2.2); daily pain (7.6 ±1.89 vs. 6.2 ±2.5); the MPQ total score (23.5 ±10.7 vs. 21.0 ±10.5)

Study	Route and form of administration	Study design	Population	Medication	Effectiveness
Safakish et al.[93] 2020 ☑	oral and inhalational routes; smoked flower or oil	prospective study 12 months	751 chronic pain patients initiating medical cannabis treatment	THC, CBD, THC:CBD	improvement in pain severity and interference was observed at 1 month and maintained over the 12-month observation period, the comparison of variables between baseline and 12 months: BPI pain interference (6.23 ±1.63 vs. 3.54 ±2.84; $p = 0.001$); and BPI pain severity (5.58 ±1.53 vs. 3.49 ±2.17; $p < 0.001$)
Ueberall et al.[94] 2019 ☑	sublingual and buccal routes; aerosol for use in the oral cavity	database study 12 weeks	800 patients with different types of chronic pain	sativex	the lowest, average and highest 24-hour pain intensity (VAS score) dropped significantly between baseline and the end of the study ($p < 0.001$ for each intensity), with ASR-9, the highest ≥50% relief rates were observed for stress (78.8%) and pain intensity (67.5%)
Kliuk-Ben Bassat et al.[95] 2022 ⊗	sublingual route; oil	crossover RCT 2 × 8 weeks	15 patients undergoing hemodialysis with chronic pain	whole-plant extract, cannabinoid extraction, placebo	differences in the BPI scores between the treatment arms did not reach statistical significance, the baseline VAS scores did not allow for comparison
Bapir et al.[96] 2023 ?	NA	cohort study 6 months	1,254 patients with chronic pain patients, with and without comorbid anxiety	CBM	in the anxiety cohort, the results for pain were inconsistent, in the non-anxiety cohort, all domains of pain improved significantly ($p < 0.05$)
Berlach et al.[97] 2006 ?	oral route; capsules	prospective study 1.5 years	20 adult patients with chronic non-cancer pain	nabilone	no significant differences between the baseline and final scores were detected for current pain intensity, and for the average and lowest pain, 45% of patients subjectively reported pain relief described as temporal, partial or extensive
Bonomo et al.[98] 2022 ?	oral route; oral solution	open-label, non-controlled dose escalation study 36 days	9 patients with chronic non-cancer pain on long-term, high-dose opioid analgesia	THC:CBD	there was no significant change in the mean pain severity, from day 17, there was a consistent reduction in the mean pain interference scores until day 30, an increase in the mean pain interference scores was observed from day 31 (after the cessation of the medication)
Capano et al.[99] 2020 ?	oral route; capsules	prospective, single-arm, cohort study 8 weeks	97 patients with chronic pain who have been on opioids for at least 1 year	THC:CBD	the PEG scale showed significant differences between the follow-up time points (6.5 (95% *CI*: 6.16–6.81), 5.9 (95% *CI*: 5.55–6.25) and 5.7 (95% *CI*: 5.31–6.12) at baseline, week 4 and week 8, respectively, $p = 0.006$), PDI showed no significant changes starting from 38.02 (95% *CI*: 35.38–40.66) at baseline, and declining to 36.40 (95% *CI*: 34.15–38.73) and 34.10 (95% *CI*: 31.61–36.58) at weeks 4 and 8, respectively ($p = 0.090$)
Gruber et al.[100] 2021 ?	NA	observational study 6 months	37 patients with chronic pain	medical cannabis	changes from baseline through 6 months: VAS (47.94 ±27.59 vs. 39.85 ±26.31; $p = 0.10$); NRS (4.56 ±2.62 vs. 3.78 ±2.42; $p = 0.10$); PAD (3.74 ±2.23 vs. 2.74 ±1.97; $p = 0.04$); PDI (26.93 ±16.36 vs. 19.15 ±13.60; $p < 0.01$)
Lynch et al.[101] 2006 ?	oral and inhalational routes	mean follow-up: 23.6 months	30 patients with chronic severe pain not controlled by traditional medical approaches	MM	93% of patients reported moderate or greater pain relief (no p-values reported)
Schubert et al.[102] 2023 ?	oral route; oral liquids, capsules granulate, or flos	database study	718 patients with chronic refractory pain, including arthritis	THC, CBD, THC:CBD	for the overall cohort on THC:CBD, the pain interference ($p = 0.007$), pain intensity ($p = 0.025$), and pain impact scores ($p = 0.023$) improved, corresponding with clinically meaningful improvement in 49 (43%), 27 (24%) and 47 (42%) participants, patients taking a CBD-dominant or THC-dominant product did not report any statistically significant improvement in any PROMIS-29 domain
Weber et al.[103] 2009 ?	oral route; capsules	retrospective study	124 patients with chronic central neuropathic pain and fibromyalgia	THC	pain intensity on VRS decreased from median 8 to median 4 ($p < 0.001$), there were differences in treatment success depending on the diagnosis

LGP – Little Green Pharma (Perth, Australia); PROMISE-29 – Patient-Reported Outcomes Measurement Information System; OR – odds ratio; BPI – Brief Pain Inventory; MPQ – McGill Pain Questionnaire; SF-MPQ-2 – Short-form McGill Pain Questionnaire 2; ASR-9 – nine-factor aggregated symptom relief score; PEG – three-item scale assessing pain intensity and interference; PDI – pain disability index; PAD – Pain and Distress scale; VRS – verbal rating scale; ⊗ – all results non-significant; ☑ – all results significant; **?** – some results significant or no statistical comparison conducted; NA – data not available.

Maintenance treatment remains being investigated. Most studies lasted only several weeks, which is insufficient for chronic pain management. Additionally, real-world evidence indicates low adherence and high treatment discontinuation rates. Horsted et al. reported that in long-term follow-up, 30% of patients discontinued treatment due to the lack of perceived analgesic effect and 7% due to the lack of funds.[88] However, the cause for treatment withdrawal remains unknown for most patients.

Discussion

The authors decided not to perform a systematic review, since they wanted to present the diversity of studies. Systematic reviews use specific types of studies and the authors wanted to present a broader approach to the topic. The studies presented in this article show a diversity of studies in terms of the composition of the substance, the route and time of its administration and, above all, the method of measuring the effect.

The goal of this review was to investigate the effectiveness of cannabis in chronic pain syndromes. The effectiveness of cannabis-based products varied across the studies. Cannabinoids were most effective in treating chronic secondary headache and orofacial pain, chronic secondary visceral pain, chronic secondary musculoskeletal pain, and chronic neuropathic pain. When qualifying a patient for cannabis treatment for pain reduction, factors including pain characteristics, comorbidities and the availability of other treatment options should be taken into account. Shared decision-making is essential to set additional treatment goals, such as reducing opiate use. Researchers recommend starting with low doses of cannabinoids and gradually adjusting them to achieve the desired therapeutic effect while minimizing adverse effects. This review revealed substantial gaps in the evidence regarding precise treatment patterns, particularly for the long-term maintenance treatment needed by patients with chronic pain.

In the present review, cannabis and CBD were found to be most effective in managing chronic secondary musculoskeletal pain, chronic secondary visceral pain and chronic neuropathic pain, which is consistent with recommendations from clinical research. However, guidelines and recommendations vary considerably across contexts due to the legal status of these medicines and the varying acceptance levels of low-quality evidence as a proof of effectiveness. The increasing popularity of cannabis and its derivatives has prompted researchers to summarize the evidence concerning their use in a recent systematic review and meta-analysis by Bell et al.,[104] and to develop clinical practice guidelines for managing chronic pain and co-occurring conditions by using these products. The authors reached several conclusions regarding the use of CBM in individuals with chronic pain. Cannabis-based medicines can be used for managing chronic pain as monotherapy, replacement therapy or adjunctive treatment, including central and peripheral neuropathic pain, to enhance pain outcomes (a strong recommendation, moderate-quality evidence). As adjunctive treatment, CBM can be used if other modalities fail to achieve an adequate response, for managing pain in individuals with multiple sclerosis (a strong recommendation, moderate-quality evidence), for fibromyalgia pain, and other chronic pain in individuals with fibromyalgia, arthritic conditions, chronic migraines, or chronic headaches (a strong recommendation, low-quality evidence).[104] The European Academy of Neurology (EAN) included medical cannabis for the management of pain in the guidelines on the palliative care of people with severe, progressive multiple sclerosis.[105] The guidelines recommend the use of any of the 3 different cannabinoid preparations (Δ9-THC, *Cannabis sativa* plant extract or nabiximols) to reduce pain in patients with severe multiple sclerosis (a weak recommendation, low-quality evidence).[105] In the clinical practice guideline from the American Society of Clinical Oncology (ASCO) on the management of chronic pain in survivors of adult cancers, medical cannabis is included in the chapter on pharmacological interventions/miscellaneous analgesics.[106] Medical cannabis or cannabinoids can be considered for use in cancer survivors experiencing chronic pain, following the careful consideration of the potential benefits and risks associated with the available formulations (a moderate recommendation, intermediate-quality evidence).[105] On the other hand, the National Institute for Health and Care Excellence (NICE) developed separate guidelines for the use of cannabis-based medicinal products, which advise against providing CBM for the management of chronic pain in adults.[107,108]

Despite the positive impact of cannabis on the treatment of pain of various origin, it is necessary to mention its side effects and risk. Evidence has suggested that cannabis may be harmful for mental, but also physical health. Side effects can be as minor as nausea, drowsiness, diarrhea, anxiety, and impaired memory and concentration. Yet, in the long run, it can lead to the deterioration of QoL, as well as mental disorders or strong addiction to cannabis.[109] Evidence suggests detrimental effects on cognition and an association with motor vehicle accidents, what can lead to injuries or death.[110] Marijuana smoke and tobacco smoke share common carcinogens, such as toxic gases, reactive oxygen species (ROS) and polycyclic aromatic hydrocarbons, which can lead to cancer.[111]

People using cannabis for chronic pain often experience a range of comorbid conditions, such as insomnia, obstructive sleep apnea (OSA) and depression. According to research, up to 54% may suffer from comorbid depression, and nearly half of patients prescribed MM (for any medical indication) report using it in order to cope with depression.[112] A study by O'Brien et al. showed that over 70% of the study sample reported at least one additional comorbid or secondary condition, and about 12.5% reported 5 or more comorbid or secondary conditions.[113] Cannabis is sometimes used as a self-medication strategy to manage these symptoms, given its potential to alleviate pain, improve sleep quality and reduce depressive symptoms.[114] However, the relationship between cannabis and comorbidities is complex, and highly dependent on the person and their specific physical and mental condition.

Availability and the legal environment determine patient access to cannabinoids, and impact both treatment patterns in patients with chronic pain and the conduct of clinical research.[115,116] The legal environment differs

between countries, affecting access to cannabis-based medicinal products, and their composition, labeling and online distribution.[117,118] In Israel, local legal regulations permit issuing a medical cannabis license to treat chronic non-cancer pain, preferably of neuropathic origin, only for patients who have unsuccessfully used conventional treatment for at least a year and have exhausted all other treatment options.[84] The approved initial monthly dose is 20 g, with concentrations of 0–24% for CBD and 0–20% for THC. Upon license renewal, the dose can be incrementally increased by 10 g per month. Cannabinoids can be administered via inhalation or as sublingual oil extracts.[84] Furthermore, using THC alone is not allowed.[92] In other countries, like Germany, medical cannabinoids were introduced for pain treatment in 2017, despite regulatory institutions not approving any of the available substances for this indication.[85] In the UK, the NICE guidelines issued in 2019 advised against the use of cannabis-based medicinal products.[108] Only patients who had already started using this treatment for pain before the guidelines were issued could continue; new patients cannot start treatment with cannabis-based medicinal products for the management of pain.[108]

The main limitation of evidence in this review is the absence of large, well-designed controlled trials. Many studies encompassed mixed patient populations, characterized not only by a high diversity of pain diagnoses and characteristics, but also by various treatment patterns and forms of CBM usage.[38,61,62] It is important to highlight that ⅓ of the studies and over half of the included patients represented diverse diagnoses. This emphasizes the necessity for more evidence from homogeneous patient groups to better inform clinicians and enable more precise recommendations. Another factor that could have potentially biased the results is the inclusion of studies that analyzed pain as a secondary outcome, focusing more on co-occurring conditions while also examining the impact of cannabinoids on pain. Such studies might be underpowered to properly determine the effectiveness of cannabinoids in pain management. Many conditions are closely linked to pain, such as spasticity in multiple sclerosis, anxiety and depression, and musculoskeletal disorders with impaired mobility. Improving co-occurring impairment may result in the alleviation of pain.[46,56,119]

In addition, the included studies show different routes of drug administration, including oils, dried herbs, gels, creams, tablets, capsules, inhalations, vaporizers, and simply smoking. Treatment regimens were not provided in relation to the route of administration. A visible gap in the studies is therefore the dependence of treatment effectiveness on the route of drug administration.

It should also be emphasized that the conducted review is a narrative review, which has its limitations. There are differences in the power of studies, heterogeneity of findings, and other factors compared to a systematic review that can be considered as limitations of the conducted review.

Conclusions

Medical cannabis can be considered an option in carefully selected patients with chronic pain syndrome for the management of chronic pain when other treatment options fail to achieve an adequate response, and when potential benefits outweigh the risks. Patients with chronic secondary headache and orofacial pain, chronic secondary visceral pain, chronic secondary musculoskeletal pain, and chronic neuropathic pain can benefit more than other groups of patients experiencing chronic pain. However, there is still a need for well-designed clinical research to establish the long-term efficacy and safety of cannabinoids.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Marta Bort https://orcid.org/0009-0002-7032-0379
Cyprian Olchowy https://orcid.org/0000-0002-6559-3973
Anna Olchowy https://orcid.org/0000-0002-5601-7173
Izabela Nawrot-Hadzik https://orcid.org/0000-0002-5797-7336
Joanna Smardz https://orcid.org/0000-0003-0004-3134
Mieszko Wieckiewicz https://orcid.org/0000-0003-4953-7143

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Review

Bibliometric analysis of the current status and trends in dental applications of glass fiber-reinforced composites from 1998 to 2022

Khalid Salman Almulhim[1,A,D–F], Shafiq Ur Rehman[2,A,C,E], Saqib Ali[3,D–F], Shakil Ahmad[4,B–D], Abdul Samad Khan[1,A,D–F]

[1] Department of Restorative Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
[2] Institute of Information Management, University of the Punjab, Lahore, Pakistan
[3] Department of Biomedical Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
[4] Central Library, Prince Sultan University, Riyadh, Saudi Arabia

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):783–795

Address for correspondence
Khalid S. Almulhim
E-mail: ksalmulhim@iau.edu.sa

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
The Authors would like to thank Prince Sultan University, Riyadh, Saudi Arabia, for their support.

Received on July 4, 2023
Reviewed on August 29, 2023
Accepted on September 3, 2023

Published online on October 31, 2024

Abstract

Over the last few years, considerable attention has been devoted to glass fiber-reinforced composites (GFRCs) in the field of dentistry. Glass fiber-reinforced composites are useful in prosthodontics, endodontics, restorative dentistry, orthodontics, and periodontics. This study considered various aspects related to GFRCs to assess the publications and citations on the subject from 1998 to 2022.

A bibliometric method of analysis was adopted to conduct the study. The relevant papers published within the established time frame were identified. A document-type filter was applied to retrieve only those results that were peer-reviewed. The most influential authors, journals, institutions, and countries were identified, as indicated by the number of citations, as well as the most frequently used keywords.

The findings of the bibliometric analysis revealed that the first article on GFRCs in the context of dentistry was published in 1998. The greatest number of papers on this subject was published in 2011 ($n = 51$), while the number of citations reached its peak in 2008 ($n = 1{,}546$). The University of Turku in Finland published the greatest number of articles, whereas Brazil was the most prolific country, producing the highest number of publications on dental fiber. Researchers from Brazil, Italy and Germany mainly collaborated with researchers from other countries, including the USA and Finland. The analysis revealed that publications of multiple authors were more likely to be cited.

Significant advancements have been made in the field of GFRCs, as demonstrated by an increased collaboration amongst different countries, organizations and investigators, which enhanced the development and progression of research related to GFRCs.

Keywords: bibliometric analysis, dentistry, biocompatible materials, glass fiber, glass fiber-reinforced polymers

Cite as
Almulhim KS, Rehman SU, Ali S, Ahmad S, Khan AS. Bibliometric analysis of the current status and trends in dental applications of glass fiber-reinforced composites from 1998 to 2022. *Dent Med Probl.* 2024;61(5):783–795. doi:10.17219/dmp/171803

DOI
10.17219/dmp/171803

Introduction

The use of fiber-based materials in biomedical applications is not a novel phenomenon. In the field of orthopedics, fiber-based materials are used in the fabrication of lower-limb prostheses,[1] reconstruction of craniofacial bone defects,[2] and implants.[3] Among the different types of fibers, such as carbon, polyaramide and high molecular weight polyethylene, glass fiber-based materials have been used for biomedical applications due to their exceptional mechanical properties, lightweight characteristics, aesthetic appeal, and compatibility with the adjacent hard and soft tissues.[4–6] Moreover, their compatibility with the polymer matrix makes them a suitable biomedical material.[7,8] For dental applications, glass fibers can be reinforced in acrylic-based polymers, such as poly(methyl methacrylate) (PMMA) and bisphenol A-glycidyl methacrylate (Bis-GMA). Additionally, both the tooth structure and the glass fiber-reinforced composite (GFRC) share analogous physical characteristics.[9] Glass fiber-reinforced composites are widely used in various dental applications, including fixed partial dentures (FPDs), restorative and endodontic post systems, periodontal splints, and fixed orthodontic retainers.[10] They were initially introduced as a reinforcement for denture base in the 1960s. Subsequently, several studies were conducted to assess the strength of GFRC,[11–13] which is currently considered an effective enhancement of dental devices, including dental base polymers. The glass fibers showed superior results in strengthening the provisional partial dentures in comparison to other fiber types.[14] Even in the modern era of dental implantology, a majority of patients opt for removable dentures to improve oral health-related quality of life.[15] Therefore, glass fibers can be utilized for the repair and reinforcement of dentures. Thermoplastic GFRCs were effectively applied in the fabrication of bonded FPDs, with the material being indirectly fabricated in a laboratory setting prior to its intraoral use. Other advantages of using glass fiber in prosthetics include the minimum invasive preparation of the abutment teeth compared to metal-ceramic and all-ceramic FPDs,[16] the lower incidence of allergic and toxic reactions associated with metal alloys, and the increased mechanical properties and time required for preparation. Glass fiber-reinforced composites are useful in aesthetic and metal-free dental and restorative applications.[10] The introduction of such material allows scientists to tailor the composite material to meet the designer's requirements and enhance its properties, thereby making it applicable to many areas of dentistry.[17]

The first non-metallic fiber endodontic post material was described in the early 1990s by Duret et al., who used the carbon-fiber reinforcement principle.[18] However, in order to improve the aesthetic outcomes, composite materials were incorporated as the main component. The FRC post systems were introduced in 1997[19] to avoid root fractures due to their modulus of elasticity being analogous to that of the dentin substrate.[20] The glass fiber posts were incorporated owing to their translucent appearance and strength.[21] Several studies have been conducted to evaluate the mechanical properties of fiber-reinforced posts, which showed wide variability in their results, mainly due to the different materials used in their construction. In summary, the factors that affect the final mechanical properties are structural density and integrity, dimensions, fiber distribution, volume fraction, voids, and the internal bond between fiber and matrix.[22,23]

The recently commercialized short glass fiber-based dental restorative composite (everX Posterior; GC Europe, Leuven, Belgium)[24] is mainly used in the restoration of large cavities in vital and non-vital posterior teeth. The randomly oriented E-glass fibers and inorganic particulate fillers used in this material provide improved toughness of the polymer matrix. The incorporation of short E-glass fiber fillers resulted in an enhanced load-bearing capacity and improved flexural strength and fracture toughness of the dental composite resin, as compared to conventional particulate filler restorative composite resin.[25] The inclusion of short fibers minimized the polymerization shrinkage stress and led to a decrease in marginal microleakage. Therefore, the positive results observed in the in vitro research that employed short glass fiber-based composite resin suggest its clinical use, as it is capable of fulfilling the requirements for ideal posterior restorations.[26] Previous research has demonstrated that the use of the GFRC wire can yield superior results in terms of patient acceptance and structural integrity.[27]

Burstone and Kuhlberg described a novel clinical application of GFRCs by applying an aesthetic connecting bar for active tooth movement.[28] This application was of significant importance due to the enhanced mechanical properties and fracture resistance under masticatory forces. In contrast, the rigid connection of teeth was considered a disadvantage, as it resulted in independent physiologic tooth movement during function. This subsequently led to the development of temporomandibular joint disorders, which can occur due to malocclusion.[29] The debonding or fracturing of the composite-based splints occurred within a few weeks or months.[30] These less rigid fiber-based composite retainers allowed for minimal tooth movements, which ultimately yielded unsatisfactory results. The recent introduction of glass fiber bundles (EverStick Ortho; Stick Tech Oy, Turku, Finland) that are pre-impregnated with a PMMA polymer has led to an improvement in the micromechanical and chemical adhesion of the splint.[31]

The inherent rigidity of the resins used in composite splints makes them susceptible to debonding failure.

The reinforcement of the resin-based composites with glass fiber has shown promising results, enabling clinicians to replace metal wires and conventional resin composites as periodontal splints with more resilient and aesthetic solutions.[32] Conservative and indirect prosthetic splinting have been readily available through the use of different types of commercial glass fiber splints, which also exhibit sufficient mechanical strength and facilitate the maintenance of proper oral hygiene.[33] In recent years, there has been a notable increase in interest surrounding the use of GFRCs in dentistry, with numerous narrative and systematic review articles having been published on the subject.[6,10,34] However, a critical analysis of these studies is essential to assess their impact on the direction of dental research. A bibliometric analysis is a method of evaluating the impact of a given scientific field. This approach has been applied to a variety of dental materials, including dental polymers,[34] composites,[35] adhesives,[36] electrospun fibers in dentistry,[37] and lithium disilicate.[38] However, our study found no bibliographic study that quantitatively assessed GFRCs. The bibliographic studies provide detailed information about subdisciplines, facilitate the organization of a field of study, and identify connections between different disciplines. Furthermore, a bibliometric analysis enables the identification of topic clusters, literature gaps and academic silos, as well as the determination of the most impactful authors and their research. In contrast to narrative literature reviews, bibliographic literature reviews use quantitative and statistical methods to achieve this goal.[39] Previous research has shown that bibliographic studies can lead to the identification of promising future research areas by identifying gaps in existing knowledge.[40]

Therefore, the aim of this study was to examine citation trends and publications in the field of GFRCs from 1998 to 2022. The other research questions addressed in this study are as follows:

- What is the pattern of authorship in the field of glass fiber research in dentistry?
- What is the extent of research conducted in the field of glass fiber research in dentistry, and how has it evolved over time?
- Which papers have been most frequently cited, and which document types are the most preferred in this field of research?
- Which authors have had the greatest influence on glass fiber research in dentistry?
- Which countries, institutions, publishers, journals, and funding agencies are the most prominent in this research area?
- Which countries and institutions are currently engaged in glass fiber research in dentistry?
- What are the key research areas and keywords in glass fiber research in dentistry?

Material and methods

Study design

The study was conducted using a bibliometric method of research analysis. This method is quantitative in nature and employs statistical tools to analyze the publications and citation trends across different fields of knowledge. Previous studies have adopted a similar bibliometric approach to map the research in their specific field.[34,41,42]

Data retrieval

The data was retrieved from the Scopus database, one of the largest indexing databases. The Scopus database was selected due to its comprehensive coverage of published scientific literature. A search strategy, accompanied by a set of inclusion and exclusion criteria, was adopted to retrieve the bibliographic records.

The following search query was carefully designed using the Boolean operators:

TITLE-ABS-KEY (“Glass fiber composite” AND “dentistry”) OR TITLE-ABS-KEY (“Glass fiber” AND “Dental Application”) OR TITLE-ABS-KEY (“Glass fiber” AND “Endodontic posts”) OR TITLE-ABS-KEY (“Glass fiber” AND “prosthodontics”) OR TITLE-ABS-KEY (“Glass fiber” AND “Fixed Prosthodontics”) OR TITLE-ABS-KEY (“Glass fiber” AND “crowns”) OR TITLE-ABS-KEY (“Glass Fiber” AND “orthodontics”) OR TITLE-ABS-KEY (“Glass fiber” AND “periodontology”) OR TITLE-ABS-KEY (“Glass Fiber” AND “Dental Splints”) OR TITLE-ABS-KEY (“Glass Fiber” AND “Core buildup”) OR TITLE-ABS-KEY (“Glass Fiber” AND “Dental Implants”) OR TITLE-ABS-KEY (“Glass Fiber” AND “Dental composites”) AND (LIMIT-TO (DOCTYPE, “ar”) OR LIMIT-TO (DOCTYPE, “re”) OR LIMIT-TO (DOCTYPE, “cp”) OR LIMIT-TO (DOCTYPE, “ch”)) AND (LIMIT-TO (SUBJAREA, “DENT”)).

Two of the investigators (SUR and SA) downloaded the data simultaneously to avoid any bias in data collection. The retrieved data was then matched, and any differences were discussed and resolved.

Eligibility criteria

A document-type filter was applied to retrieve solely the peer-reviewed results. Original papers, reviews, editorials, book chapters, and conference papers were included in the study. Documents that did not undergo the peer-review process were excluded.

Tools used

The data was analyzed and visualized using Microsoft Excel 2016 (Microsoft Corporation, Redmond, USA), biblioshiny (https://www.bibliometrix.org/home/index.php/layout/biblioshiny) and VOSviewer (https://www.vosviewer.com).

Results

Figure 1 illustrates the number of authors (AU) engaged in glass fiber research within the field of dentistry, along with their total number of publications (TP) and total number of citations (TC). The data indicates that researchers preferred to work collaboratively. Six- and five-author studies were the most common trend, respectively. The greatest number of studies were carried out through the collaboration of 6 authors (n = 130). The highest number of citations were received from the publications authored by 5 individuals (n = 3,702), followed by papers published by 4 authors (n = 3,063). Interestingly, the trend of publications exhibited a decline with an increase in the number of authors, such as 9 and 10. The number of citations received by publications authored by a single or 2 authors was relatively high, i.e., 847 and 1,542, respectively. The total number of publications for these authors was 16 and 38, respectively.

The line graph in Fig. 2 illustrates the number of publications and citations in glass fiber research. The data reveals an asymmetrical growth of both publications and citations in the studied field. The first publication on the subject was released in 1998. The number of publications steadily increased until 2003. Subsequently, a non-linear behavior was observed until 2022. In 2011, the greatest number of publications was observed (n = 51), which reached its highest citation number in 2008 (n = 1,546). Papers published from 2017 to 2022 received a reasonable number of citations. For example, papers published in 2017, 2018 and 2019 received 158, 254 and 180 citations, respectively.

Table 1 presents a list of the 10 most highly cited papers,[21,27,43–50] accompanied by the names of their authors,

Fig. 1. Relationship between the number of authors (AU), the total number of publications (TP) and the total number of citations (TC) in the field of glass fiber research in dentistry from 1998 to 2022

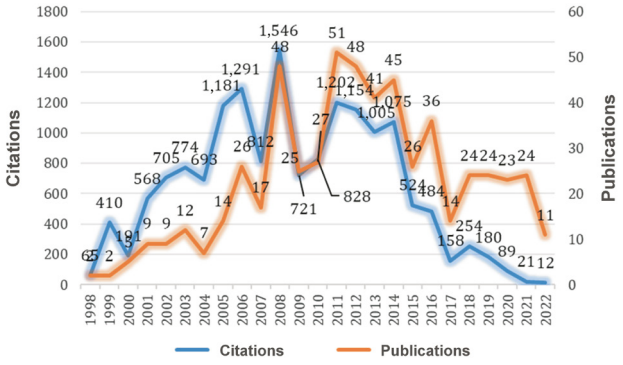

Fig. 2. Evolution in the number of publications and citations in the field of glass fiber research in dentistry from 1998 to 2022

the year of publication, the source of publication, TC, and the average number of citations per year. The paper published in 2002 by Akkayan and Gülmez received the highest number of citations, with a yearly average of 21.05 citations.[43] The paper was published in the Journal of Prosthetic Dentistry. The findings showed that the most frequently cited papers were published between 1999 and 2005,[21,43–47,49,50] with the exception of 2 papers published in 2013[27] and 2014,[48] which received 151 and 175 citations, respectively. The study by Sarkis-Onofre et al. exhibited the highest yearly average number of citations, namely 21.88 citations.[48]

Table 2 provides a list of the authors, along with the year of their first and most recent publications. Additionally, it provides the number of publications each author has contributed as a single author, first author, and in other capacities. The TP and TC values for these authors were tabulated. Vallittu PK was the most prolific author with 35 publications and 2,159 citations. Following Vallittu PK, Ferrari M contributed 32 publications and received 1,531 citations. Among the most prolific authors, Vallittu PK was responsible for 4 studies alone.

Figure 3 presents the TP and TC values for original papers and reviews. Original papers were the most prevalent form of publication, comprising approx. 98% of the research output. Reviews were the other prominent document type. Original papers received the highest share of total citations (n = 15,240), while reviews received the highest average number of citations per document (n = 58), outperforming all other document types.

Figure 4 shows the countries engaged in collaborative research related to glass fiber. Brazil, Italy and Germany demonstrated the greatest degree of collaboration in various studies related to dental fiber and other related areas. These 3 countries demonstrated a significant level of collaboration with the USA. With regard to the Arab region, Saudi Arabia had demonstrated the highest degree of collaboration (Fig. 4). Among the countries of Finland, Turkey, the USA, China, and Japan, Finland and Turkey showed high inter-collaborative publication activity.

Table 1. Most frequently cited papers in the field of glass fiber research in dentistry from 1998 to 2022

No.	Authors and the year of publication	Title	Journal	TC	Yearly average
1	Akkayan and Gülmez 2002[43]	Resistance to fracture of endodontically treated teeth restored with different post systems	The Journal of Prosthetic Dentistry	421	21.05
2	Vallittu 1999[44]	Flexural properties of acrylic resin polymers reinforced with unidirectional and woven glass fibers	The Journal of Prosthetic Dentistry	336	14.61
3	Lassila et al. 2004[45]	Flexural properties of fiber reinforced root canal posts	Dental Materials	280	15.56
4	Lanza et al. 2005[46]	3D FEA of cemented steel, glass and carbon posts in a maxillary incisor	Dental Materials	224	13.18
5	Cormier et al. 2001[47]	In vitro comparison of the fracture resistance and failure mode of fiber, ceramic, and conventional post systems at various stages of restoration	Journal of Prostodontics	176	8.38
6	Sarkis-Onofre et al. 2014[48]	The role of resin cement on bond strength of glass-fiber posts luted into root canals: A systematic review and meta-analysis of in vitro studies	Operative Dentistry	175	21.88
7	Goracci et al. 2005[49]	Evaluation of the adhesion of fiber posts to intraradicular dentin	Operative Dentistry	168	9.88
8	Goracci et al. 2005[50]	The adhesion between prefabricated FRC posts and composite resin cores: Microtensile bond strength with and without post-silanization	Dental Materials	165	9.71
9	Garoushi et al. 2013[27]	Physical properties and depth of cure of a new short fiber reinforced composite	Dental Materials	151	16.78
10	Bateman et al. 2003[21]	Fibre-based post systems: A review	British Dental Journal	144	7.58

TC – total number of citations.

Table 2. Most prolific authors in the field of glass fiber research in dentistry from 1998 to 2022

No.	Author	Year of publication		Author			TP	TC
		first	last	single	first	other		
1	Vallittu PK	1998	2021	4	0	31	35	2,159
2	Ferrari M	2001	2019	0	4	28	32	1,531
3	Lassila LVJ	2001	2021	0	3	27	30	1,254
4	Soares CJ	2008	2020	0	5	16	21	786
5	Valandro LF	2005	2020	0	3	17	20	300
6	Goracci C	2001	2015	0	5	14	19	1,153
7	Naumann M	2005	2019	0	8	7	15	690
8	Rosentritt M	2000	2019	0	2	12	14	540
9	Kern M	2003	2020	0	0	11	11	415
10	Monticelli F	2002	2012	0	4	7	11	643

TP – total number of publications.

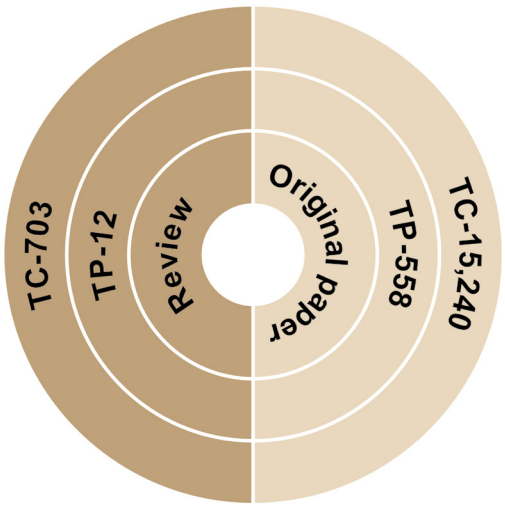

Fig. 3. Preferred document types in the field of glass fiber research in dentistry from 1998 to 2022

Table 3 lists the publishers with the highest number of publications. The TP, TC, and average citation per publication (TC/TP) for these publishers are presented. It was found that Mosby Inc. was the most prolific publisher, with 26 publications, followed by Elsevier Inc., with 21 publications. The highest number of citations per publication was recorded for these 2 publishers. The mean number of citations per document for these publishers was 35.

Table 4 lists the institutions that sponsored 4 or more studies. The TP funded by agencies, TC, and TC/TP are presented. The highest number of studies were funded by the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) (n = 13). The publications financed by the Ministry of Science, Technology and Innovation of Brazil were cited with the best average of 67 citations per publication. The highest number of citations were received by the publications funded by the National Council for Scientific and Technological Development (CNPq) (n = 404).

The word cloud shown in Fig. 5 provides the most frequently used keywords in glass fiber research in dentistry. The terms "fiber post", "fracture resistance", "bond strength", and "endodontically treated teeth" were the most prevalent in the studies relevant to dental fiber. Other notable terms include "resin cement", "adhesion" and "fiber-reinforced composite."

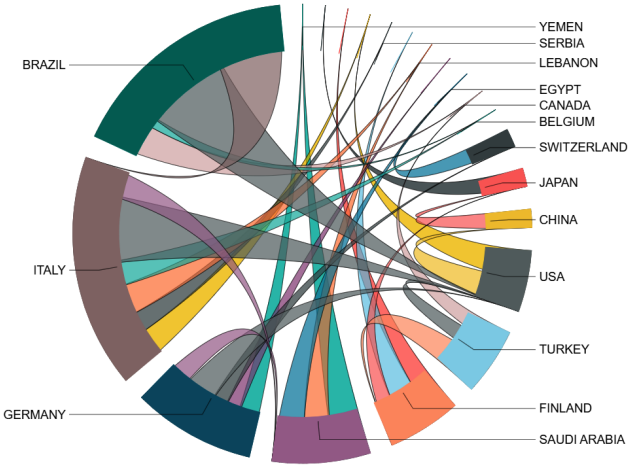

Fig. 4. Countries engaged in collaborative research in the field of glass fiber research in dentistry from 1998 to 2022

Table 3. Top 10 publishers in the field of glass fiber research in dentistry from 1998 to 2022

No.	Publisher	TP	TC	TC/TP
1	Mosby Inc.	26	920	35.38
2	Elsevier Inc.	21	736	35.05
3	Jaypee Brothers Medical Publishers Pvt. Ltd.	17	100	5.88
4	Indiana University School of Dentistry	17	381	22.41
5	Associação Brasileira de Divulgação Científica	13	179	13.77
6	Quintessence Publishing Company, Ltd.	13	149	11.46
7	Japanese Society for Dental Materials and Devices	11	84	7.64
8	Wolters Kluwer Medknow	10	25	2.50
9	National Library of Medicine (MEDLINE)	8	19	2.38
10	Faculdade de Odontologia de Bauru	8	160	20.00

TC/TP – average citation per publication.

Table 4. Top 10 funding agencies of publications in the field of glass fiber research in dentistry from 1998 to 2022

No.	Funding agency	TP	TC	TC/TP
1	Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES)	13	136	10.46
2	National Council for Scientific and Technological Development (CNPq)	12	404	33.67
3	Fundação de Amparo à Pesquisa do Estado de Minas Gerais	9	351	39.00
4	São Paulo Research Foundation	9	292	32.44
5	Ministry of Science, Technology and Innovation of Brazil	5	333	66.60
6	Academy of Finland	4	177	44.25
7	Japan Society for the Promotion of Science	4	26	6.50
8	Ministry of Education, Culture, Sports, Science and Technology	4	26	6.50
9	National Institute of Dental and Craniofacial Research (NIDCR)	4	249	62.25
10	Finnish Funding Agency for Innovation (TEKES)	4	242	60.50

Figure 6 illustrates the evolution of terminology in glass fiber research in dentistry over 2 time periods: 1998–2012; and 2013–2022. The terms "flexural strength", "fiber posts" and "fracture load" were the most frequently used terms from 1998 to 2012. The data indicates that a number of new terms emerged during the final 10 years of the study period. In the period between 2013 and 2022, researchers demonstrated a preference for the terms "fibre posts" and "fracture resistance". The terms "fiber post", "flexural strength" and "fiber-reinforced composite" remained equally popular, and were used throughout the study period.

Table 5 presents a list of the journals in which the researchers in this field preferred to publish their research. The Journal of Prosthetic Dentistry published the greatest number of studies (n = 50), followed by Dental Materials (n = 48). Papers published in Dental Materials received the highest number of citations (n = 2,951).

Figure 7 reveals the most productive countries. Brazil was the most prolific country in terms of the number of publications on dental fiber. Brazilian researchers published 182 papers that were cited 3,857 times. The USA published 66 articles, while Germany and Italy published 64 papers each. Saudi Arabia was the only country from the Arab region to be included among the top publishers

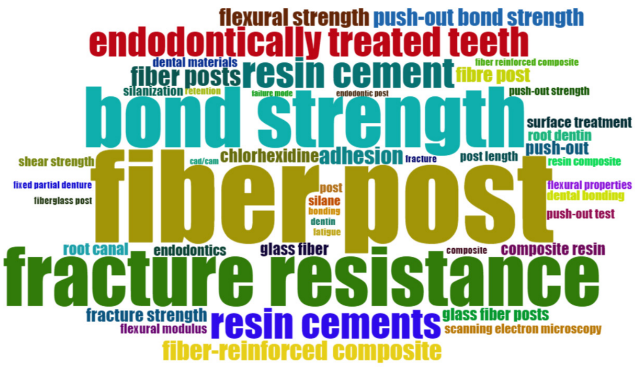

Fig. 5. Word cloud showing the keywords most commonly used in publications related to glass fibers in dentistry

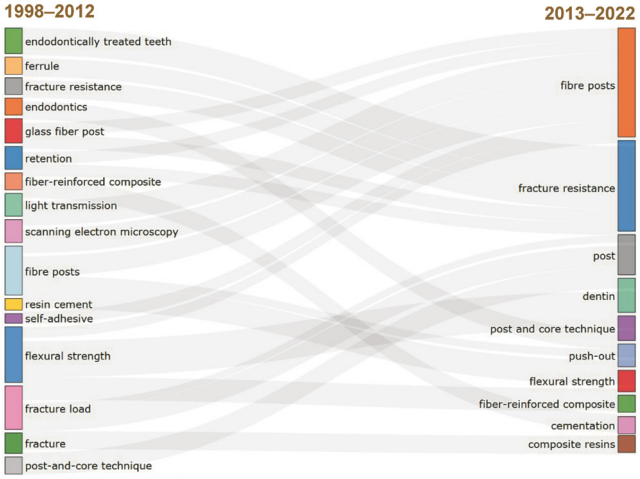

Fig. 6. Evolution of terms used in the field of glass fiber research in dentistry from 1998 to 2022

Table 5. Journals most frequently selected for publication in the field of glass fiber research in dentistry from 1998 to 2022

No.	Publisher	TP	TC
1	The Journal of Prosthetic Dentistry	50	2,148
2	Dental Materials	48	2,951
3	Operative Dentistry	38	1,290
4	Journal of Dentistry	30	1,084
5	The Journal of Endodontics	28	1,538
6	The Journal of Adhesive Dentistry	28	427
7	Dental Materials Journal	24	330
8	International Endodontic Journal	24	560
9	General Dentistry	21	134
10	Journal of Prosthodontics	19	498

worldwide, with 21 papers and 413 citations. It is noteworthy that Finland had 39 publications, yet the number of citations was 2,201.

Figure 8 reveals the most active institutions worldwide that have published research on GFRCs in the context of dentistry. The University of Turku (Finland) was the most prolific institution, publishing the highest number of articles (n = 39). This university also received the greatest number of citations (n = 2,201). Subsequently, the University of Siena (Italy) and the University of São Paulo (Brazil) demonstrated comparable performance, with 34 and 28 publications, respectively, and 1,642 and 855 citations, respectively.

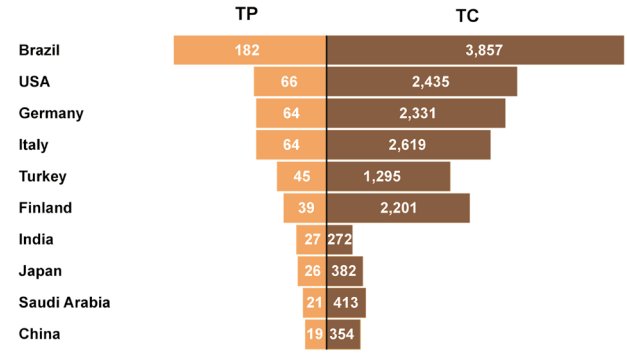

Fig. 7. Countries with the highest number of publications and citations in the field of glass fiber research in dentistry from 1998 to 2022

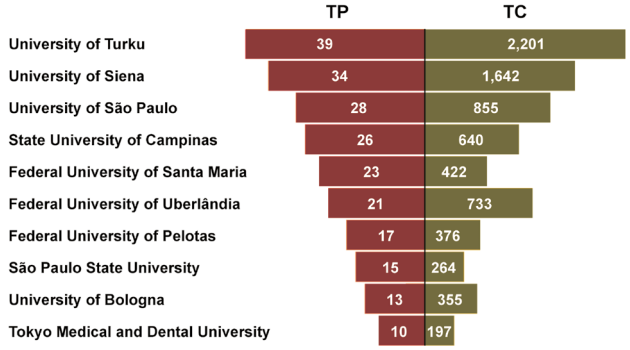

Fig. 8. Institutions with the highest number of publications and citations in the field of glass fiber research in dentistry from 1998 to 2022

Figure 9 shows the evolution of trending topics between 1998 and 2022. In the early years of the study period, the term "fibers" was mostly used. Conversely, in recent years, the terms "dental prosthesis", "polyetheretherketone (PEEK)", "dentin bonding agents", and "glass-fiber post" were the most common. The terms "fiber post", "bond strength" and "fracture resistance" were among the most frequently used between the years 2012 and 2016.

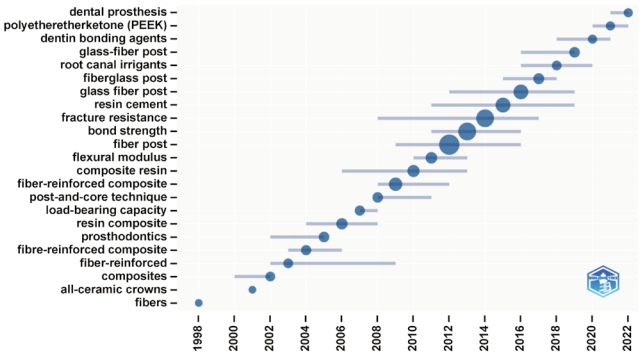

Fig. 9. Frequently used terms in the field of glass fiber research in dentistry from 1998 to 2022

Discussion

Glass fiber-reinforced composites have been incorporated into dentistry due to their superior strength and biocompatibility compared to conventional strategies.[1,51] The recent advancements in the field of glass fibers have significantly improved the mechanical efficacy of these materials.[52] A bibliometric analysis involves the application of various statistical and mathematical methods to determine the number of documents and associated bibliographical data, evaluate author contributions, identify the countries and institutions with the highest scientific production, and examine the themes and types of papers published.[53,54] The present study identified the most influential authors, journals, institutions, and countries, along with the most frequently used keywords, as indicated by the number of citations. The bibliometric analysis revealed that the first 2 publications on GFRCs were published in 1998. These papers evaluated the pattern and composition of the glass fibers and discussed the clinical application of these composites in fixed prosthodontics. The 2 papers were published in Dental Clinics of North America (first quartile (Q1)) and the Journal of Prosthodontics (Q1), and received 21 and 44 citations, respectively.[51,52] Among these, the book chapter by Belvedere analyzed the principle and technique for increasing strength by the reinforcement of fibers, and discussed the chairside technique for single-visit bridges for teeth.[55] The most notable publication was that of Vallittu,[56] which examined the woven structure and components present in the GFRCs and compared their weaving pattern using

a scanning electron microscope and a spectrometer. The study concluded that E-glass plays a beneficial role due to its hydrolytic stability over time.

This study evaluated the number of publications in the field of glass fiber research in dentistry per year (1998–2022). Overall, a non-linear behavior was observed in terms of publications and citations in Scopus-indexed journals. The asymmetrical growth of publications and citations in the studied field showed that in 2011, the greatest number of articles was published (n = 51), followed by a decline in the number of subsequent publications. After the sudden drop in the number of publications, a similar pattern was observed in citation data. It was difficult to determine the cause of this sudden decrease. However, it is assumed that the lack of innovations and researchers' interest in GFRC-based dental restorative materials may have contributed to this state of affairs. Since its introduction in dentistry, the majority of materials have been based on E-glass fibers, with a similar range of resin matrices. There has been a paucity of experimental research conducted in this area. The surface treatments of glass fibers are utilized in dentistry.[57,58] Furthermore, limited studies have been reported concerning the inclusion of bioactive particles or surface grafting of the glass fibers with nanoparticles.[59–61] Glass fiber-reinforced composites have been used for all major dental applications. However, additional marketing is necessary, as the cost of the glass fiber-based orthodontic retainers, periodontal splints and endodontic posts is comparatively higher than that of contemporary materials. It is possible that dentists are still unconvinced regarding this material. A similar trend was observed in another bibliometric study,[62] where, after 2017, there was a decrease in the number of publications related to glass fiber-based endodontic posts.

The bibliometric analysis revealed that Brazil was the leading country in terms of scientific publications related to glass fiber, with a substantial number of articles published on an annual basis. These findings were in accordance with the results of a previously conducted bibliometric analysis.[63] The prominent funding agencies in Brazil are the CNPq, CAPES, and Funding Agency for Studies and Projects (FINEP). These institutions are responsible for providing financial support to a range of organizations, educational universities and the training of new researchers in various universities. Moreover, the São Paulo Research Foundation, associated with the State Department of Economic Development, Science and Technology (SDECT), receives approx. 560 million US dollars annually for research purposes. This funding facilitates scientific and technological advancement by supporting research projects across a range of fields.[64,65] The current bibliographic analysis revealed that the CAPES and CNPq funded 13 and 12 papers, respectively, in this domain, and received a high number of citations.

The USA was the second largest contributor to scientific publications. This was due to the substantial financial resources allocated to research by the National Institute of Dental and Craniofacial Research (NIDCR) and the existence of a vast scientific community.[66–68] Similarly, the results of the current analysis demonstrated that the NIDCR provided funding for GFRC-based research, which was also highly cited. Additionally, Germany, Italy and Finland also made substantial contributions. From the Arab regions, Saudi Arabia made a significant contribution, as evidenced by the publication of 21 papers, which were cited 413 times. This can be attributed to the fact that in 2016, Saudi Arabia launched Saudi Vision 2030, a program aimed at supporting Saudi Arabia in becoming one of the top 10 countries in the Global Competitiveness Index.[69] Among the different parameters, the top ranking was achieved by increasing the number of publications by institutions from Saudi Arabia. Moreover, various resources were provided to increase the level of research productivity, including the provision of facilities and the allocation of approx. 1.6 billion US dollars to enhance the number of research publications in highly prolific journals.[70] Furthermore, authors from Saudi Arabia have collaborated with researchers from Egypt, Yemen and Lebanon.

The institutions occupying the highest ranking positions were from Finland, Italy and Brazil. The University of Turku, Finland, published the highest number of papers and received the highest number of citations. This was attributed to the funding provided by the Academy of Finland, the European Commission, and the Finnish Funding Agency for Innovation (TEKES).[71,72] Moreover, the research groups comprising Vallittu and his colleagues, who are affiliated with the University of Turku, contributed significantly to this domain.

The majority of studies on GFRCs were published in highly influential core journals. The exact criteria used for describing a core journal are not entirely agreed upon. Usually, the term "core" is applied to journals publishing the highest number of articles within a given domain. However, the journal's impact factor has also been used as a benchmark.[73–75] Frequently, authors prioritize journals with a high impact factor over those with a high readership.[76] This is in line with Bradford's law, which suggests that about one-third of publications in a particular field are more often published in the core journals.[77,78] A similar pattern was observed in the presented bibliometric analysis, where authors demonstrated a preference for publishing papers in journals with a high impact factor. The Journal of Prosthetic Dentistry (Q1, impact factor (IF) = 4.3) published the highest number of papers (n = 50), followed by Dental Materials (Q1, IF = 4.6), Operative Dentistry (Q1, IF = 2.2), and the Journal of Dentistry (Q1, IF = 4.8), which published 48, 38 and 30 papers, respectively. These Q1 Scopus journals showed a significant increase in their impact factors over the past 7 years. For example, in comparison to the preceding year, these journals demonstrated an increase in impact factor of 0.88, 0.36, 0.42,

and 0.65, respectively, in 2021.[74] This clearly illustrates the importance of the journal by calculating the number of selected citations in previous years, which intrigues enthusiastic researchers who usually prefer to publish in high-impact factor journals.

Regarding the citations, articles published earlier generally attain a greater number of citations, compared to those published later.[79] Nonetheless, the position of highly cited papers may vary over time.[80] In this analysis, Dental Materials received the highest number of citations (2,951), while the Journal of Prosthetic Dentistry and the Journal of Endodontics received 2,148 and 1,538 citations, respectively. In addition, Mosby Inc. was recognized as the most prolific publisher, with 26 papers published, followed by Elsevier Inc., which published 21 papers.

The type of publication is another factor that affects the number of citations a paper receives. In general, review articles are cited more frequently than other article types, as they provide a rich source of information regarding the existing scientific literature and available data within a particular domain.[81,82] On the contrary, in this analysis, original articles received more citations than review articles. It was observed that articles that are easily accessible online were more frequently cited. Additionally, the year of publication, the number of authors and the quality of the research paper were found to be significant factors.[83]

The most highly cited paper on the topic (421 citations) was written by Akkayan and Gülmez and published in 2002 in the Journal of Prosthetic Dentistry.[43] The study evaluated the fracture resistance of the endodontically treated teeth by using posts composed of quartz fiber, titanium, zirconia, and glass fiber. The findings of this study indicated that teeth restored with quartz fiber demonstrated higher fracture resistance compared to other restoration materials.[43] The 2nd most highly cited paper (336 citations) was written by Vallittu and published in the Journal of Prosthetic Dentistry in 1999.[44] The study incorporated glass fiber reinforcements (Stick and Stick Net) into the heat-cured denture base, denture-based polymers and temporary fixed partial dentures. After the preparation of the specimens, a three-point test was conducted to evaluate the flexural modulus and transverse strength. Glass fiber-reinforced material provided promising results in enhancing the flexural strength of the materials.[44] The 3rd most highly cited article was written by Lassila et al. and published in Dental Materials in 2004.[45] It received 280 citations and was co-authored by Vallittu, a renowned researcher affiliated with the University of Turku, Finland. This scientific group contributed significantly to the field of glass fiber research in dentistry. This article evaluated the potential of fiber-reinforced composite posts from various brands and diameters. Subsequently, a three-point bending test was applied to evaluate the modulus and the flexural strength of fiber-reinforced composite-based posts. The study concluded that commercially manufactured fiber-reinforced composite (FRC) posts had lower flexural strength than the discretely polymerized FRC material.[45] The 4th most highly cited paper (224 citations) was written by Lanza et al. and published in Dental Materials in 2005.[46] This paper assessed the stress distribution pattern within the dentinal and cemental layer in a root canal-treated maxillary central incisor. Carbon, glass and steel posts were subjected to occlusal load and evaluated using finite element analysis (FEA). The results demonstrated that carbon and glass fiber posts exhibited higher tensile and fatigue strength, and could be easily bonded with the root canal.[46]

The 5th most highly cited paper (176 citations) was written by Cormier et al. and published in the Journal of Prosthodontics in 2004.[47] The study evaluated the strength to resist fracture, the failure mode, and the ease of removing failed post systems for 6 post systems at 4 different stages. A variety of procedures and tests were performed at each stage. The study revealed that fiber-based posts offer greater benefits than the conventional posts system.[47]

Keywords are an essential element of a scientific publication. In performing a literature search, the use of relevant keywords allows to retrieve significant results, as opposed to using phrases or sentences. Keywords have been recognized to act as a code, which facilitates the retrieval of pertinent scientific articles.[84] Moreover, the results of the current bibliometric analysis indicate that the keywords assist in determining the trend of research publications within this domain.[34] These frequently used keywords aid investigators and researchers in identifying relevant articles related to glass fiber.

The term "fiber-reinforced composite" was the most frequently used keyword between 2002 and 2009. The use of FRC in dentistry was first documented in the 1960s. However, it was not until the early 1990s that researchers gained interest in this material, leading to a notable increase in the number of publications on the subject.[85] The material was extensively examined as an endodontic post system in direct restorations and fixed partial dentures.

A number of countries and institutions have launched various interdisciplinary research projects with the aim of promoting global research and detecting and resolving complex problems through a comprehensive understanding of the subject matter.[86,87] Policymakers and researchers have also studied and examined the benefits of interdisciplinary collaboration, which not only creates integrative knowledge but also substantially improves the quality of research and communication among researchers.[88,89] Similarly, collaboration among multiple authors was identified in the current analysis owing to the rising interest of researchers in the domain of GFRCs. These collaborative research projects among international authors are encouraged as they frequently result in publications that attain higher impact and citations.[90,91] This emphasizes the growing expectation for

more extensive collaboration and joint research projects in the future.

Numerous papers with the highest number of citations had 4–10 authors, many of whom were affiliated with different research institutions and countries. Publications with a greater number of authors receive a higher number of citations. This phenomenon can be attributed to 3 factors: citations being received from external sources; self-citations; and higher visibility, as these publications are more likely to have a higher impact and quality. Furthermore, publications comprising authors from various disciplines are more likely to attain citations from a variety of sources.[92] This further highlights the value of collaboration among countries, organizations and investigators in advancing research related to GFRCs. In addition, Vallittu PK, who is affiliated with the University of Turku in Finland, is a highly cited author who has made a significant contribution to this research area and has collaborated extensively with researchers from around the globe.

The present bibliometric analysis had some limitations. As mentioned earlier, only the Scopus database was used to obtain bibliometric data. Therefore, some GFRC-related publications may have been overlooked. The articles selected for this study were limited to those written in English, thus it is possible that some GFRC-related publications may have been omitted. Moreover, even though a bibliometric analysis was performed, the authors did not conduct a quality assessment of the included studies. The number of citations a paper received allowed for a quantitative evaluation of the scientific influence of publications within a certain domain. Notably, a scientific publication with a high citation count does not necessarily indicate high quality, as the number of citations can be manipulated. In addition, the total citation count included self-citations, given the absence of available methodology.

Despite these shortcomings, the most recent data available from the Scopus database presents a comprehensive overview of recent research trends regarding GFRCs. It would be beneficial for future research projects to perform analysis using other databases such as Web of Science, Google Scholar and PubMed.

Over the course of the evaluated time period, considerable attention was given to the use of GFRCs in biomedical applications, which was followed by a recent trend of applying these materials in the dental field. It is well established that these materials offer improved clinical results, are highly biocompatible and possess excellent mechanical and aesthetic properties. Accordingly, many narrative and systematic review articles related to this topic have been published. The present bibliometric analysis evaluated their impact on applied science in the field of dentistry. However, concerns were raised regarding the use of only one type of glass fiber, i.e., E-glass fiber. The cost of S-glass fibers is high, and their service life is relatively short. Similarly, the long-term efficacy of glass fiber-based bridges is questionable. Further research is required to improve the physical and mechanical properties of glass fiber-based dental materials. It is recommended that additional restorative materials should be manufactured based on glass fibers. The surface properties of the glass fibers should also be improved. New challenging studies should address the orientation of fibers, as most of the fibers can be used only in one direction due to their anisotropic behavior.

Conclusions

It was concluded that, within the limitations of this study, the obtained bibliometric analysis provided detailed information regarding the trend of research publications on GFRCs and their citations from 1998 to 2022. A remarkable increase in the number of publications was observed in 2011, which marked the peak in the number of papers published during the period under review. Brazil was identified as the leading country in terms of research output, while the University of Turku was identified as the most prolific institution. In addition, a trend of collaborative research projects with multiple authors was identified. The journal Dental Materials published papers that were highly cited, while the keywords "fiber-reinforced", "composite resins", "fracture resistance", and "glass fiber posts" were frequently used. This bibliometric study aimed to provide future research directions for investigators and researchers in order to identify any missing gaps in the field of GFRCs. It is anticipated that this bibliographic study will provide researchers with direction, allowing them to find potential research groups with whom they can collaborate and to ascertain which institutions are mostly involved in research related to glass fibers. Furthermore, it will enable them to identify the area of research that will be of interest to other researchers (conclusion based on the analysis of citations). The present bibliographic study revealed that glass fiber posts and, more recently, the application of short fibers in dental composites have emerged as areas of interest among researchers. Nevertheless, this field requires further investigation.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Khalid Salman Almulhim https://orcid.org/0000-0003-4163-7342
Shafiq Ur Rehman https://orcid.org/0000-0002-8169-0132
Saqib Ali https://orcid.org/0000-0002-2421-0753
Shakil Ahmad https://orcid.org/0000-0001-7775-4917
Abdul Samad Khan https://orcid.org/0000-0002-2165-800X

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Research letter

Cardiorespiratory and circadian clock markers in intensive care unit patients

José Manuel Jiménez-Pastor[1,2,B,C,F], Francisco Rodríguez-Cortés[1,2,B,C,F], Pablo López-Soto[1,2,A–F], Luna López-Coleto[1,2,B,C,F], Miguel Meira e Cruz[3,4,C–F]

[1] Department of Nursing, The Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Spain
[2] Department of Nursing, Pharmacology and Physiotherapy, University of Cordoba, Spain
[3] Sleep Unit, Centro Cardiovascular da Universidade de Lisboa (CCUL@RISE), School of Medicine, Lisbon, Portugal
[4] European Sleep Center, Lisbon, Portugal

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(5):797–801

Address for correspondence
Miguel Meira e Cruz
E-mail: mcruz@medicina.ulisboa.pt

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on February 14, 2024
Reviewed on July 9, 2024
Accepted on July 23, 2024

Published online on October 31, 2024

Abstract

Background. Biological synchronized rhythmicity is a critical physiological process. The lack of synchronized rhythms, mainly those showing a circadian basis, like sleep, the heart rate (HR) and arterial blood pressure (BP), often leads to several organic challenges, usually associated with adverse outcomes.

Objectives. The aim of the study was to investigate whether the intensive care unit (ICU) environment favors clock genes and cardiorespiratory changes.

Material and methods. A total of 22 critically ill patients (16 males; 72.73%) with a mean age of 60.82 ±20.07 years and well-established cardiovascular conditions were selected from ICU. Blood samples were obtained, and total RNA was isolated and reverse-transcribed into complementary DNA (cDNA). A quantitative polymerase chain reaction (qPCR) was performed to assess the target gene expression levels. The urinary concentration levels of melatonin (MEL) were assessed. The heart rate, BP (systolic – SBP, diastolic – DBP and mean – MBP) and the oxygen saturation (SpO_2) levels were assessed as continuous variables.

Results. The urinary MEL and Brain and muscle Arnt-like protein-1 (BMAL1) levels were shown to have a non-linear relationship with HR (coefficient (coef): 2.318, $p = 0.032$; coef: 2.722, $p = 0.006$, respectively) and SBP (coef: 1.000, $p = 0.008$; coef: 2.000, $p = 0.037$, respectively), with an explanatory power of up to 50.3% and 39.7% of the HR and SBP variability, respectively. Melatonin, but not BMAL1, was also shown to have a non-linear relationship with MBP (coef: 1.000, $p = 0.007$), with an explanatory power of up to 31.3% regarding the MBP variability. The HR and SBP oscillatory dynamics was shown to be related to changes in the genetic expression of BMAL1 and the urinary MEL concentrations. To a lower degree, MEL also impacted the variation of MBP.

Conclusions. Our results suggest that not only are circadian functional matrices crucial for the dynamics of vital parameters in critically ill patients, but also that routinely assessed cardiovascular parameters like HR and BP may constitute important markers for the circadian timing system function. These parameters are easy to assess and have a relevant prognostic value regarding recovery outcomes, as well as the morbidity and mortality rates in ICU.

Keywords: ICU, circadian disruption, clock genes, chronodisruption, cardiorespiratory functions

Cite as
Jiménez-Pastor JM, Rodríguez-Cortés F, López-Soto P, López-Coleto L, Meira e Cruz M. Cardiorespiratory and circadian clock markers in intensive care unit patients. *Dent Med Probl.* 2024;61(5):797–801. doi:10.17219/dmp/191537

DOI
10.17219/dmp/191537

Introduction

Biological synchronized rhythmicity is a critical physiological process. The lack of synchronized rhythms, mainly those showing a circadian basis, like sleep, the heart rate (HR), and arterial blood pressure (BP), often leads to several organic challenges, usually associated with adverse outcomes. Sleep itself, as an independent regulator of many crucial body functions, should preferentially occur with minimum interference to optimize its role in structural and functional recovery and regeneration. Patients mostly benefit from the optimal circadian rhythmicity and sleep, which translates into better prognoses and reduces hospital discharge times.[1] Meanwhile, the hostile intensive care unit (ICU) environment is typically linked to a negative impact on both the circadian timing system and sleep regulation centers, with important implications in the recovery of critically ill patients.[1–3] Despite some of the most common disruptors being identified,[1] it is largely unknown how they interact with the molecular matrix of the body's internal time and, therefore, whether they might have a major contribution to a persistent deleterious role associated with the worsening of the main clinical condition, or increased morbidity and mortality.[4] We, therefore, hypothesized that the circadian timing system and cardiorespiratory functions may interact in such a way that the disrupted circadian rhythmicity, typically occurring in ICU patients, would influence the crucial cardiovascular and respiratory parameters, like HR, BP and the oxygen saturation (SpO_2) levels.

Material and methods

The present study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Provincial Research Ethics Committee of Cordoba (approval No. 277; ref. 3878). All patients were older than 18 years and gave their consent to participate voluntarily in the study.

A total of 22 critically ill patients (16 males; 72.73%) with a mean age of 60.82 20.07 years and well-established cardiovascular conditions (45.45% – cardiovascular surgery; 27.27% – ST-elevation myocardial infarction (STEMI); 4.55% – non-rheumatic mitral valve disease; 4.55% – out-of-hospital cardiac arrest; and 18.18% – cardiogenic shock) were selected from all the patients admitted with these characteristics to ICU. Specifically, accepting an alpha risk of 0.05 for a precision of ±0.15 units in a two-tailed test, with an estimated standard deviation (*SD*) of 0.35 and an estimated replacement rate of 0.10, a random population sample of 22 subjects was required, assuming that the available population in the cardiovascular pathology area of ICU during the recruitment period was 400 subjects. Data collection was performed from the 3rd quarter of 2020 until the end of the 2nd quarter of 2021. The participants were required to be over 18 years old and be treated in ICU for at least 48 h. Pregnant women, patients admitted to the restricted access modules, or those with scores above 13 in the Acute Physiology and Chronic Health Evaluation II (APACHE II) scale at baseline were excluded.

Prior to inclusion in the study, the patients were provided with an information sheet, and written informed consent was obtained from all participants. If the patients were unable to give their consent due to their clinical condition, consent was requested from their families. An A-weighting filter was applied during the different work shifts, and the following data on the patients' physiological state was collected using a physiologic monitor (IntelliVue MP5; Philips, Amsterdam, the Netherlands): HR; the respiratory rate (RR); BP; and SpO_2. Human whole blood samples were obtained from the 22 patients at 4 time points along 2 circadian cycles (8 h, 13 h, 18 h, and 23 h). Total RNA was isolated, and each blood sample was subsequently reverse-transcribed into complementary DNA (cDNA). A quantitative polymerase chain reaction (qPCR) was performed to assess the target gene expression levels (Brain and muscle Arnt-like protein-1 – BMAL1, circadian locomotor output cycles kaput – CLOCK). The urinary concentration levels of melatonin (MEL) were assessed at 12 consecutive time points along 2 circadian cycles (8 h, 10 h, 12 h, 14 h, 16 h, 18 h, 20 h, 22 h, 00 h, 2 h, 4 h, and 6 h). The heart rate, BP (systolic – SBP, diastolic – DBP and mean – MBP) and the SpO_2 levels were assessed as continuous variables. A proficient researcher recorded the physiological variables at 15-minute intervals, and these measurements were also programmed into the physiologic monitor for continuous monitoring. The descriptive data of the studied patient group is gathered in Table 1.

To model the non-linear trends of the vital parameters of the patients in ICU, the generalized additive mixed models (GAMMs) were used following a twofold rationale: a) the variation of the repeated measurements within and between the subjects should be taken into account, as vital parameters are repeatedly assessed over time for the same patient; and b) the non-linear effect of time on vital parameters could be captured using smoothing splines. This type of model is frequently used to study both the variation of the repeated measurements within and between the subjects with random effects, as well as the non-linear effects of covariates on the response variable with the smooth function of the covariate. Likewise, these flexible smoothing techniques help to capture small increases and decreases in the outcome variable during the follow-up period.

Table 1. Descriptive data of the studied patient group (*N* = 22)

Characteristic		Data
Age [years] *M* ±*SD*		60.82 ±20.07
Gender *n* (%)	M	16 (72.73)
	F	6 (27.27)
Clinical diagnosis *n* (%)	cardiovascular surgery	10 (45.45)
	STEMI	6 (27.27)
	non-rheumatic mitral valve disease	1 (4.55)
	out-of-hospital cardiac arrest	1 (4.55)
	cardiogenic shock	4 (18.18)
HR [bpm] *M* ±*SD*		94.57 ±1.55
SBP [mmHg] *M* ±*SD*		115.5 ±2.34
DBP [mmHg] *M* ±*SD*		61.53 ±1.50
MBP [mmHg] M ±SD		79.51 ±1.54
SpO_2 [%] *M* ±*SD*		97.95 ±0.44
MEL [ng/mL] *M* ±*SD*		5.22 ±2.15
Length of stay in ICU [days] *M* ±*SD*		31.00 ±17.38
Hospital discharge *n* (%)		11 (50.00)
GCS score *Me* +*SEM*		1.0000 ±0.0499
RASS score *Me* +*SEM*		–2.0000 ±0.0329
White light [lx] *M* ±*SD*		28.36 ±47.31
Blue light [lx] *M* ±*SD*		8.18 ±15.60
Infrared light [lx] *M* ±*SD*		3.32 ±11.28
Sound [dB] *M* ±*SD*		64.09 ±9.88
Epinephrine [mcg/kg/min] *M* ±*SD*		0.1500 ±0.1617
Norepinephrine [mcg/kg/min] *M* ±*SD*		0.2858 ±0.3747
Midazolam [mL] *M* ±*SD*		4.522 ±2.465
Propofol [mL] *M* ±*SD*		9.931 ±5.080
Fentanyl [mL] *M* ±*SD*		1.813 ±0.949

M – mean; *SD* – standard deviation; *Me* – median; *SEM* – standard error of the mean; HR – heart rate, SBP – systolic blood pressure; DBP – diastolic blood pressure; MBP – mean blood pressure; SpO_2 – oxygen saturation; MEL – melatonin; ICU – intensive care unit; GCS – Glasgow Coma Scale; RASS – Richmond Agitation-Sedation Scale; M – male; F – female; STEMI – ST-elevation myocardial infarction.

Results

Both MEL and BMAL1 (but not CLOCK) were shown to have a non-linear relationship with HR (coefficient (coef): 2.318, $p = 0.032$; coef: 2.722, $p = 0.006$, respectively) and SBP (coef: 1.000, $p = 0.008$; coef: 2.000, $p = 0.037$, respectively), with an explanatory power of up to 50.3% and 39.7% of the HR and SBP variability, respectively. Melatonin, but not BMAL1, was also shown to have a non-linear relationship with MBP (coef: 1.000, $p = 0.007$), with an explanatory power of up to 31.3% regarding the MBP variability. The HR and SBP oscillatory dynamics was shown to be related to changes in the genetic expression of BMAL1 and the urinary MEL concentrations. To a lower degree, MEL also impacted the variation of MBP (Fig. 1 and 2).

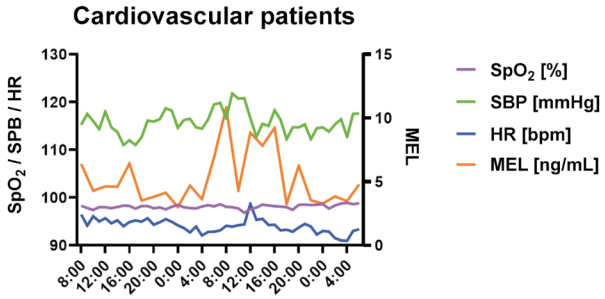

Fig. 1. Distribution of oxygen saturation (SpO_2), systolic blood pressure (SBP), the heart rate (HR), and the urine melatonin (MEL) concentration during the 2 circadian cycles (48 h) of patient follow-up in the intensive care unit (ICU). Urine samples were collected every 2 h, but the X-axis shows intervals of 4 h to facilitate visualization. The average values for each time point are provided

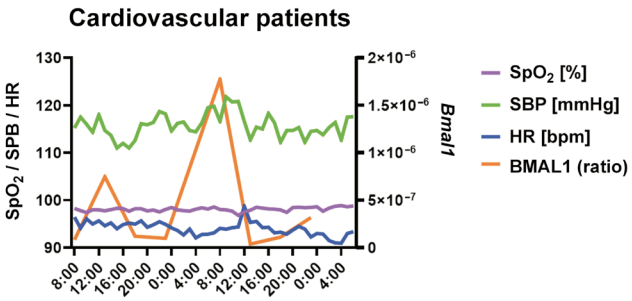

Fig. 2. Distribution of oxygen saturation (SpO_2), systolic blood pressure (SBP), the heart rate (HR), and the expression of BMAL1 mRNA (the ratio of the number of *Bmal1* copies to the number of copies of the housekeeping gene, in this case, *actin*) in human whole blood samples during the 2 circadian cycles (48 h) of patient follow-up in the intensive care unit (ICU). The X-axis shows intervals of 4 h to facilitate visualization. The average values for each time point are provided

Discussion

Together, the results suggest that not only are circadian functional matrices crucial for the dynamics of vital parameters in critically ill patients, but also that routinely

assessed cardiovascular parameters like HR and BP may constitute important markers for the circadian timing system function. These parameters are easy to assess and have a relevant prognostic value regarding recovery outcomes, as well as the morbidity and mortality rates in ICU. Still, it is crucial to recognize that while patient-related factors are undoubtedly significant contributors to sleep disruption, the influence of the ICU environment should not be underestimated, and may be influencing the genetic and molecular aspects of circadian dysrhythmias, and chronobiological disruption.

From this work, a significant relationship between certain molecular markers of the biological clock and circadian rhythms and cardiovascular parameters like HR and BP was noted in ICU patients, implying that disruption in circadian rhythms can have a direct effect on these cardiovascular parameters, and may lead to the worsening of the ICU patient's condition. Circadian disruption is common in ICU patients; several studies demonstrate that environmental conditions in ICU, especially excessive noise, particularly during patients' rest periods, and exposure to artificial light at times of the day when they should be in darkness,[5] can disrupt patients' sleep and circadian rhythms, in turn worsening and delaying recovery. Indeed, emerging evidence indicates a link between disruption in circadian rhythms and poorer clinical prognoses.

Our findings may be of particular importance with regard to the subgroups of critical patients, like those with sleep dysfunctional complaints, or manifestations like insomnia or obstructive sleep apnea (OSA), both quite prevalent in ICU,[6,7] impacting the circadian timing system[8] and causing cardiometabolic impairment.[9] Furthermore, as they frequently co-occur (as in the case of Co-Morbid Insomnia and Sleep Apnea (COMISA)), exacerbating the cardiovascular and associated metabolic risk,[9,10] such pathological dynamics would plausibly have a negative effect on the circadian rhythmicity, sleep maintenance and general outcomes of this critical population. Interestingly, clock-related neuronal PAS domain protein-2 (NPAS2) has been recently found to be lower in OSA patients (with shortened sleep time) as compared to otherwise healthy individuals.[11] As NPAS2 has been shown to be implicated in metabolic abnormalities, such as insulin resistance, it might be questioned whether the circadian-mediated cardiometabolic risk in OSA and perhaps insomnia could, in part, be explained by circadian-induced genetic changes. Still, other pathways and mechanisms could also support such interplay between circadian changes, sleep disturbance and the poor cardiometabolic outcomes often observed in ICU, such as some electrolytes, like calcium (Ca) and magnesium (Mg). From our findings, one could speculate about the putative contribution of Mg to the circadian disruption-related cardiometabolic risk in OSA patients. Some arguments behind this hypothesis are: 1) there is a well-established 24-hour cycle in the Mg flux[12]; 2) recent studies have found a lower Mg concentration in hypertensive OSA patients[13]; and 3) it is accepted that Mg is implicated in the metabolic dysfunctional status, therefore participating in the development of metabolic disorders.[14] The knowledge of such interactions would increase awareness and likely benefit future research, as well as provide methods of adopting care that would favor the positive outcomes and survival rates of critically ill patients.

Felten et al. explored whether preserving or reinstating circadian regularity could enhance outcomes for patients in critical care settings, such as ICU and neonatal ICU.[15] Strategies encompass adjustments to the environment to align with natural light-dark cycles and interventions such as medication timing and feeding schedules.[15] The same authors proposed that advanced machine learning algorithms might enhance the analysis of clinical routine data on rhythmic patterns, using tools like cosinor analysis, therefore leading to a better understanding of how chronodisruption may affect important clinical outcomes. These innovations may enable real-time rhythmicity assessment and evaluation in the ICU environment, improving both the prediction of critical outcomes and therapeutic decisions.[15] These molecular markers of circadian rhythms could also be used as biomarkers to establish the presence or absence of circadian dysregulation, and somehow support a prediction for patient recovery rates. Specifically, MEL could be an ideal candidate,[16] as it is one of the central components of circadian rhythm regulation, and, as we have seen in our work, it has a non-linear relationship with HR, SBP and MBP. Melatonin may also be implicated in deregulatory mechanisms among distinct sleep disorders and cardiovascular diseases[17]; therefore, studying its levels in ICU patients could provide valuable information, obtained non-invasively, about the circadian status and sleep-associated performance of patients. The use of MEL could be studied as a possible intervention for ICU patients with circadian dysregulation, to attempt to restore normal circadian cycles, and greatly promote patient improvement and recovery.[18] In addition to restoring circadian rhythms, and consequently positively affecting physiological parameters, the use of MEL as therapy may have a beneficial effect on other common complications of ICU patients, such as sepsis,[19] one of the leading causes of death following traumatic injury.[20]

Limitations

There are certain limitations to our study, the main one being the high diversity and variability of the included conditions. This methodological constraint, which is related to the high heterogeneity of the recruited patients, was assumed from the initiation of the study design, since a more stringent criterion would critically affect the sample size. On the other hand, while these results do not allow generalizations to any particular model of dis-

turbance, they point to a major hypothesis converging on distinct pathophysiological pathways commonly occurring in critical patients and, therefore, of great clinical interest. In the future, studies following the same research hypothesis should try to overcome this drawback.

Conclusions

In conclusion, a non-linear relationship was found between MEL and the clock gene *Bmal1* with HR and SBP, and between MEL and MBP, indicating that the dysregulation of clock gene expression and MEL due to the hostile ICU environment may lead to disturbances in the cardiovascular variables of ICU patients.

Ethics approval and consent to participate

The present study was approved by the Provincial Research Ethics Committee of Cordoba (approval No. 277; ref. 3878). All patients gave written informed consent to participate voluntarily in the study.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

José Manuel Jiménez-Pastor https://orcid.org/0000-0003-1965-6045
Francisco Rodríguez-Cortés https://orcid.org/0000-0002-0490-7860
Pablo López-Soto https://orcid.org/0000-0002-1046-6686
Luna López-Coleto https://orcid.org/0009-0008-9116-5033
Miguel Meira e Cruz https://orcid.org/0000-0001-6076-0878

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